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VOL. 92 APRIL 1, 1930 No. 3

THE USE OF CONSTANT GLUCOSE INJECTIONS FOR THE STUDY OF INDUCED VARIATIONS IN CARBOHYDRATE METABOLISM

III. The Fate of the Retained Sugar under Normal Conditions and after Epinephrin and Insulin

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From the Laboratories of Physiology in the Harvard Medical School

Received for publication October 23, 1929

The use of a method involving the intravenous administration of glucose at constant rates to cats uniformly anesthetized by means of amytal has been justified by experiments reported previously (1). It was shown that glycosuria appears in the course or an hour or two if the administration exceeds an average rate of about 350 mgm. glucose per kilogram body weight per hour; that within two or three hours it and the blood-sugar concentration undergo variations of a minor degree, especially near the beginning of the injection, but tend to remain constant for long periods of time unless the anesthesia is markedly irregular, and that induced influences known to be capable of modifying the rate of utilization of glucose are reflected by corresponding marked changes in the glycosuria and, to a lesser extent, in the hyperglycemia. Thus the total retention of sugar, taken as the combined storage and combustion, may be measured quantitatively hour after hour as the difference between the supply and excretion. After equilibrium between blood and tissue concentrations of sugar have been established, it would seem reasonable to consider that the retained sugar represents the total utilization, as originally asserted by Woodyatt (2).

The actual fate of the retained sugar has received but scant attention so far. Boothby and Sandiford accounted for about one-quarter of it by oxidation in one of Woodyatt's experiments (3). Boyd, Hines and Leese (4) found consistent increases in the respiratory quotient, frequently exceeding unity, under similar conditions, but did not measure the protein

[1] Medical Fellow of the National Research Council.

metabolism and therefore were unable to estimate the increased oxidation quantitatively. It is true, of course, that the fate of single doses of glucose has been investigated thoroughly. The methods employed by Cori and Cori (5) and Lesser et al. (6) lead to satisfactory agreement between the amounts of sugar administered and recovered. Data obtained in that manner, however, could be of little value in interpreting investigations which employ constant injections because single doses result in continually changing sugar concentrations and consequent variable fates. They show only that in a general way the oxidation and storage of glucose are increased ultimately when it is present in excess. Furthermore, they involve calculations based on assumed volumes of circulating fluids. The studies conducted in Dale's laboratory (7) are more pertinent in this connection. They show that when glucose is given continuously at approximately constant rates, the glucose disappearing may be accounted for, with fair accuracy, as the sum of that oxidized and that stored as glycogen. But even these data are not applicable to the present problem because the excretion factor was ordinarily eliminated by ligation of the kidneys and the experiments were performed in eviscerated, spinal animals.

In the present experiments, therefore, respiratory data have been obtained in animals receiving glucose at constant rates intravenously. They show that a large part of the retained sugar is oxidized, that in certain unrecognized conditions there may be considerable transformation into fat, and that ordinarily the minor irregularities in glucose excretion which occur after the preliminary period of adjustment of concentration may be accounted for by changes in the utilization.

Methods. Most of these have been described in detail previously (1). Briefly, female cats were fed 200 grams milk and 20 grams lactose daily for 3 to 7 days prior to use. They were then uniformly anesthetized by means of a preliminary dose of 50 to 70 mgm. amytal per kgm. and a subsequent, constant intravenous supply of about 3 mgm. per kgm. per hour. Only the data obtained under uniform anesthesia were considered acceptable. Glucose was administered intravenously, usually in solutions of about 10 per cent strength, at rates (which were constant within limits of 1 per cent) between 0.5 and 1.0 gram per kgm. per hour. The urine was collected in half-hourly or hourly specimens and analyzed for sugar by the method of Folin and Berglund (8) and for total nitrogen according to Kjeldahl. The blood sugar was determined hourly by the newer method of Folin (9). In the experiments involving the use of epinephrin and insulin Parke, Davis & Company's adrenalin in 1–1,000 solution and Lilly's U-40 insulin, respectively, were employed.

The expired air was collected in a carefully counterbalanced spirometer of the Tissot type, leading through airtight flutter valves to a tracheal cannula. Continuous collections were made, the air from the first part of

each period being used to wash out the spirometer once or twice. That from the last part was measured, its volume corrected to standard conditions of temperature and pressure and a portion of it analyzed in duplicate in an apparatus of the Haldane type. The sample analyzed represented a mixture of the air expired during about one-half of the period under observation, some fifteen to thirty minutes, depending upon the ventilation, the size of the animal, the length of the period and the rate of metabolism.

CALCULATIONS. The respiratory values employed in the calculation of glucose oxidation are as follows: 774 cc. CO_2 and 966 cc. O_2 per gram protein (Loewi, 10); 746 cc. CO_2 and O_2 per gram glucose or 1.34 grams glucose per liter CO_2 and O_2 (DuBois, 11); respiratory quotient of fat 0.707.

A simple method has been worked out for calculating the glucose oxidation at intermediate non-protein respiratory quotients. It permits a calculation of glucose oxidized directly from the values for non-protein CO_2 and O_2 and avoids the necessity of interpolating in the Zuntz-Schumberg-Lusk (12) or similar tables. In the following explanatory calculations glucose oxidation is expressed in grams and all values for CO_2 and O_2 in cc. Reducing these equations

$$\text{non-protein } O_2 = \text{glucose } O_2 + \text{fat } O_2$$

and

$$\text{non-protein } CO_2 = \text{glucose } CO_2 \text{ (or } O_2) + 0.707 \times \text{fat } O_2$$

simultaneously to their simplest form, the value for glucose CO_2 (or O_2) is found to be $3.417 \times$ non-protein $CO_2 - 2.415 \times$ non-protein O_2. Substituting this value in the equation

$$\text{glucose oxidation} = \frac{\text{glucose } CO_2 \text{ (or } O_2)}{746}$$

and simplifying, the result is the formula

$$\text{glucose oxidation} = 0.00458 \times \text{non-protein } CO_2 - 0.00324 \times \text{non-protein } O_2.$$

The method adopted for calculating the amount of glucose transformed into fat is purely arbitrary. Lusk (13) finds fair agreement between the observed and calculated heat production when the calculations for the latter process involve the assumption that each liter of carbon dioxide eliminated in excess of a non-protein respiratory quotient of unity corresponds to the conversion of 4.6 grams glucose into 1.7 grams fat with production of 1.09 calories. Thus each gram of glucose forming fat would correspond to 217 cc. "extra" non-protein carbon dioxide. On the basis

of the simplest theoretical formula for this process (Bleibtrau, Lusk, DuBois, Richardson, 14) the value would be 250 cc. if the small quantity of oxygen involved was disregarded as insignificant. In the present calculations the value assigned by Lusk was employed arbitrarily, i.e.,

$$\text{glucose (grams) converted to fat} = \frac{\text{non-protein } CO_2 - \text{non-protein } O_2 \text{ (cc.)}}{217}$$

It is recognized that calculations of this process could not be expected to give more than a rough idea of the extent of fat formation from glucose. It is admittedly doubtful whether any known method would do so. Insofar as they do give a rough idea they are of service, however, and probably any error involved would at least be consistent. It is worth noting that the results calculated in this manner appear to be consistent and within the limits of what might be expected to occur under the circumstances.

RESULTS. *Simultaneous determination of glucose oxidation and excretion with a constant intravenous supply.* The glucose excretion and oxidation were measured simultaneously in at least a dozen uncomplicated experiments for varying periods of time up to nine hours. The results justify the following generalization. When the intravenous supply is maintained uniformly at a rate sufficient to cause abnormal glycosuria, the oxidation and excretion both increase at first, the latter as a rule more rapidly than the former; after the third or fourth hour, although minor variations may occur in one or the other, their sum is more constant than either taken alone (charts 1 to 4). Their absolute values depend primarily upon the rate of administration, and probably also upon the conditions existing in the different individual animals, such as depth of anesthesia and previous nutritional state. In relation to the supply rate, however, the ultimate sum of the glucose accounted for represents usually from 70 to 92 per cent of the supply, in the majority of instances from 70 to 77 per cent. This type of response occurred in all cases except the three mentioned below so that charts 1 and 2 (experiments 111 and 112) may be regarded as typical of the group. An example of the numerical data is reproduced in table 1 (experiment 112).

Experiment 54 (chart 3) is unusual in that the non-protein respiratory quotient was maintained at a level indicating continuous formation of fat for several hours. It is one of only two experiments in the series in which this phenomenon occurred. A calculation of the quantity of glucose suffering this fate, based on the empirical method described above, shows that the sum of the three processes (oxidation, conversion to fat, and excretion) in this instance accounts for a constant proportion of the supply after the third hour, just as it does when only two processes are involved, and that the sum corresponds perfectly with the usual 70 to 90 per cent recovery.

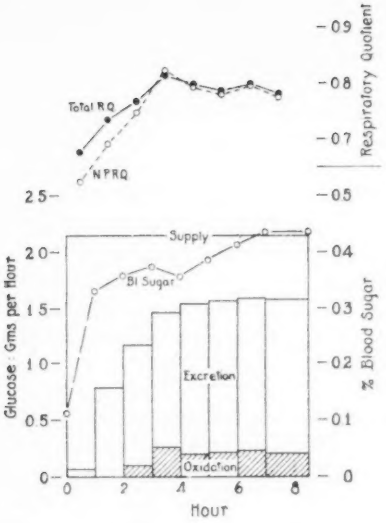

Chart 1

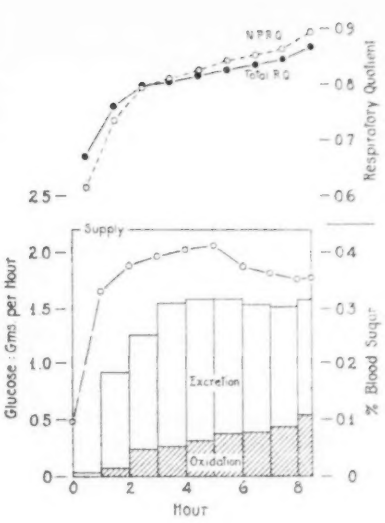

Chart 2

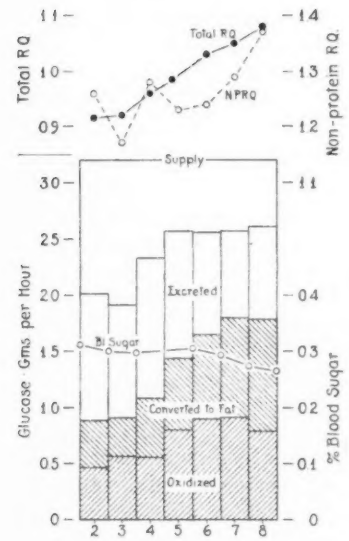

Chart 3

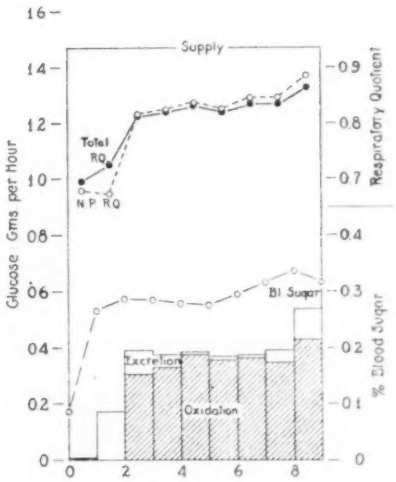

Chart 4

Chart 1. Experiment 111. Cat 318, weight 2.61 kgm. Glucose supply: 21.2 cc. 10.1 per cent solution or 2.1½ grams per hour = 0.82 gram per kgm. per hour.

Chart 2. Experiment 112. See table 1.

Chart 3. Experiment 54. Cat 262, weight 3.10 kgm. Glucose supply: 30.7 cc. 10.4 per cent solution or 3.20 grams per hour = 1.03 grams per kgm. per hour.

Chart 4. Experiment 108. Cat 315, weight 1.81 kgm. Glucose supply: 15.1 cc. 9.75 per cent solution or 1.47 grams per hour = 0.81 gram per kgm. per hour.

Experiment 108 (chart 4) is the other exceptional response mentioned. Here, in spite of appreciable fluctuations in the oxidation, the excretion varied between 11 and 88 mgm. per hour, with the result that the sum of the two was remarkably constant from the third to the eighth hours inclusive, but represented only about one-quarter of the supply instead of the usual three-quarters. Then in the last hour both values increased suddenly to levels well above those maintained previously.

This phenomenon introduces the question of the fate of that portion of the supply which is unaccounted for. During the first two hours of the

TABLE 1

Oxidation and excretion of glucose when the intravenous supply is constant (see chart 2)

Experiment 112: Cat 319, weight 2.40 kgm. Diet milk and lactose only for 3 days previously.

Glucose supply: 21.8 cc. 10.1 per cent solution or 2202 mgm. per hour = 0.92 gram per kgm. per hour.

HOUR	BLOOD SUGAR*	URINE NITR.	RESPIRATORY EXCHANGE						Sugar oxidized	SUGAR EXCRETED	TOTAL SUGAR ACCOUNTED FOR
			CO_2		O_2		R.Q.				
			Total	NP	Total	NP	Total	NP			
	per cent	*mgm.*	*cc.*	*cc.*	*cc.*	*cc.*			*mgm.*	*mgm.*	*mgm.*
0	0.098										
0–1	0.330	38.9	535	346	797	563	0.670	0.616	0	34	34
1–2	0.377	55.2	668	401	879	546	0.760	0.734	67	851	918
2–3	0.392	47.6	707	477	888	690	0.796	0.794	238	1020	1260
3–4	0.405	48.4	679	445	842	550	0.807	0.810	258	1290	1550
4–5	0.411	47.3	705	476	863	577	0.817	0.826	312	1280	1590
5–6	0.377	45.9	723	501	873	596	0.828	0.841	365	1220	1590
6–7	0.363	44.0	705	492	843	577	0.836	0.852	382	1160	1540
7–8	0.351	40.0	715	522	845	604	0.846	0.864	434	1090	1520
8–9**	0.353	36.4	742	566	853	634	0.869	0.893	540	1040	1580

* Taken at end of period.

** Observations one-half hour only. Calculated on hourly basis.

injection a portion of the retained sugar undoubtedly serves to increase the concentration of free sugar in the blood and other body fluids up to the level maintained subsequently, indicated roughly by the concentration in the blood. Calculations based on an assumed "active volume" (Burn and Dale, 15) show that the greatest quantity thus accountable could be, however, only about one-quarter of the supply. After comparative constancy of the blood sugar is established, the accumulation of free sugar is probably negligible. This leaves but one possibility, the only other known fate of glucose in the body, namely, polymerization as glycogen.

Under somewhat similar conditions Best, Dale, Hoet and Marks (7)

have been able to strike a satisfactory balance between the supply of glucose and the sum of that polymerized and oxidized. Inasmuch as excretion was eliminated by kidney ligation in their experiments the data are not strictly comparable, but they substantiate the belief that the only other possible destination of the sugar injected in the present experiments is glycogen, and that storage as a hypothetical phosphoric compound (Embden) cannot be verified by analysis, even with an excessive insulin supply (16).

Inaccuracies in the methods of analysis employed might conceivably give rise to a discrepancy between the supply and the glucose accounted for, since they involve three different types of standards, but an error as great as 25 per cent from this source seems beyond the range of possibility even with the most careless investigator. Hence it may be concluded reasonably that the missing glucose after the blood-sugar concentration becomes constant represents that fraction which is polymerized. If this is true it is obvious that the rate of glycogen formation may be measured quantitatively, hour after hour in the intact animal, in this indirect manner.

The exceptional experiment 108 (chart 4) is in accord with this view, as are others with insulin and epinephrin to be described presently. Prior to this experiment the animal was offered the usual ration of milk and sugar but did not eat and was obviously thin and weak when the injection was started. Thus it had been fasting for at least a week. Presumably its glycogen reserves were exceptionally low, and thus the high rate of glycogen formation occurring in preference to oxidation and excretion would be explained. According to this explanation sufficient glycogen accumulated during the first eight hours of the injection to make up this need and not until then did the excretion and oxidation suddenly show signs of approaching their usual values. The subsequent behavior in this experiment would have been interesting. It was interrupted before these features were appreciated.

It should be reëmphasized that constant findings can be obtained by this method only when the anesthesia is maintained uniformly at a moderate depth. If it deepens, the oxidation and excretion of sugar fall off appreciably, and if it lightens, irregularities occur such as those described in the first paper of this series (1). It seems unlikely that the unusual response obtained in experiment 108 could be due to the anesthesia, however, because it was satisfactory, judged by the usual signs, and because the disturbance noted at the end of the experiment was too abrupt to be due to a change in the depth of the anesthesia. In this case, as in all others reported, the data would have been discarded if any noticeable variation in anesthesia had occurred.

With or without the experiment in question the fact remains, nevertheless, that with a constant supply exceeding the tolerance limit the observed

oxidation and excretion of sugar eventually constitute a constant fraction of the supply, and if the missing sugar is considered to be polymerized, the rate of glycogen formation ultimately becomes constant also under the specific conditions employed. Recognition of this fact is important as a control for the observations which follow.

Effects of epinephrin and insulin. The findings in the control periods, fore and after, in a number of experiments with epinephrin and insulin

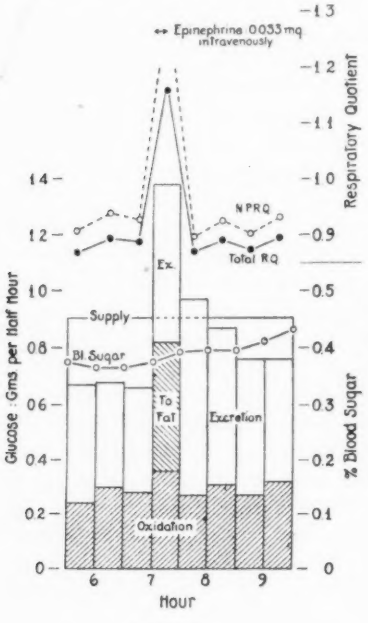

Chart 5

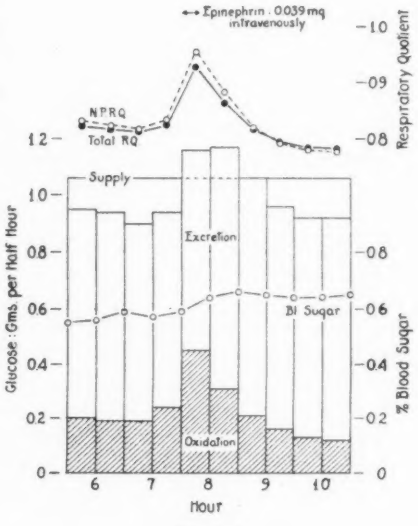

Chart 6

Chart 5. Experiment 104. Cat 311, weight 2.21 kgm. Glucose supply: 17.9 cc. 10.1 per cent solution or 1.81 grams per hour = 0.82 gram per kgm. per hour. Epinephrin: 0.033 mgm. intravenously during first quarter of eighth hour of injection.

Chart 6. Experiment 105. Cat 312, weight 2.60 kgm. Glucose supply: 21.0 cc. 10.1 per cent solution or 2.12 grams per hour = 0.82 gram per kgm. per hour. Epinephrin: 0.039 mgm. intravenously during third quarter of eighth hour of injection.

correspond with the results of the uncomplicated experiments described above. In experiments 104 and 105 (charts 5 and 6) observations were not made until equilibrium had been established, i.e., after $5\frac{1}{2}$ hours of injection. Then for $1\frac{1}{2}$ and 2 hours, respectively, the glucose oxidized and excreted together accounted for the customary 70 to 90 per cent of the supply, and the missing sugar, considered to be deposited as glycogen, was

consequently constant in amount. With these findings for control, epinephrin, given intravenously for 15 minutes at a rate of 0.001 mgm. per kgm. per minute, resulted in immediate increases in both excretion and apparent oxidation, followed in an hour or two by resumption of the approximate levels existing before the epinephrin, serving for control after-periods.

It is not possible to form an opinion concerning the immediate effect of epinephrin on the rate of glucose oxidation from the data obtained. There is no question but that the carbon-dioxide elimination is promptly increased, so calculations based on the observed findings consequently indicate an accelerated rate of sugar combustion. How much of this is due to blowing-off of carbon dioxide and how much to increased production from metabolic sources is a question which probably could not be answered finally even with knowledge concerning the behavior of the carbon-dioxide combining power of the blood. In experiment 104 (chart 5) the findings in the period in which epinephrin was given suggest considerable blowing-off, because obviously fat formation could not begin and stop suddenly in this fashion. Yet there was no compensatory retention after the epinephrin effect had worn off. In experiment 105 (chart 6) the carbon-dioxide elimination was less abruptly increased, but the average sugar oxidation for the six periods following epinephrin (including the one in which it was injected) was only slightly greater than that of the fore periods, indicating some subsequent retention of carbon dioxide.

The specific calculations employed admittedly may result in artefacts due to the uncontrollable factor in question. The writers feel that an interpretation of increased sugar oxidation after epinephrin is scarcely justified by the present data, or at least if an increase does occur, its magnitude is less than that indicated by the calculated results.

On the other hand, there can be no doubt that the excretion of sugar is augmented by epinephrin. Even if the combustion were considered to be unchanged the sum of the sugar burned and excreted would still exceed the supply at the height of the epinephrin action in both of these experiments as well as in all others not reported. Experiments to be described in the next paper show that the excretion alone may exceed the supply for eight hours and longer if epinephrin is supplied continuously.

The mechanism responsible for this phenomenon is undoubtedly the same as that producing epinephrine hyperglycemia under ordinary conditions. The abruptness of the response indicates that glycogen must be mobilized at an increased rate. Cori and Cori (17) have pointed out that another factor must be involved, however, since the hyperglycemia may be prolonged. They present data which show that since the liver does not contain enough glycogen to supply sugar for hours in excess of the normal ability to utilize it, the utilizing ability must be subnormal, chiefly,

perhaps, because glycogen synthesis in the muscles is inhibited. They find support for this view in the fact that the arterio-venous difference in sugar concentration may be diminished in epinephrin hyperglycemia. Hence, although liver glycogenolysis may be increased as well, the prolonged hyperglycemia caused by epinephrine in suitable dosage could not occur unless the normal capacity for utilizing sugar was diminished simultaneously. Inasmuch as combustion is not decreased, glycogen synthesis must be. As a matter of fact, experiments to be reported in the next paper of this series show that prolonged epinephrin administration may also

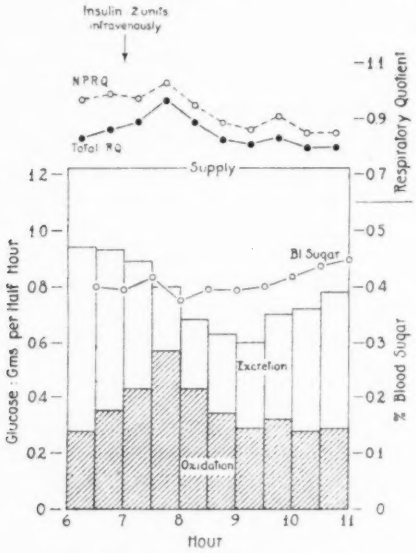

Chart 7. Experiment 106. Effect of insulin on the utilization of injected glucose. Cat 313, weight 3.00 kgm. Glucose supply: 24.1 cc. 10.1 per cent solution or 2.43 grams per hour = 0.81 gram per kgm. per hour. Insulin: 2 units intravenously at end of sixth hour of injection.

suppress sugar oxidation completely. The extra sugar appearing in the urine under the present conditions may be considered to originate in liver glycogen but to be excreted because it cannot be utilized peripherally.

Experiment 106 (chart 7) is one of two identical experiments showing the effect of a single small dose of insulin on the oxidation, glycogen storage and excretion under these conditions. Although in this case a subsequent control period was not quite realized, the tendency is clear. The apparent result is an increase of glucose combustion which lasted only about 1½ hours, whereas the effect on the storage of glucose, interpreted as that

unaccounted for, increased more gradually and outlasted the effect on combustion by at least 2½ hours. If it is conceded that the missing sugar was stored as glycogen, this demonstration affords a new conception of the time relations existing between accelerated glycogen storage and oxidation after insulin. The failure of the blood-sugar concentration to change appreciably in spite of the marked reduction in glycosuria is another illustration of the fact, noted previously (1), that the glycemic concentration does not necessarily determine the degree of glycosuria.

SUMMARY

1. When glucose is administered intravenously at constant rates high enough to cause abnormal glycosuria, after the first three or four hours a constant proportion of it (usually about three-quarters under the present conditions) can be accounted for by oxidation and excretion, and occasionally also by conversion to fat. Two implications may be made from this fact:

a. Glycogen storage eventually proceeds at a constant measurable rate.

b. After the initial period of adjustment of free sugar concentration in the tissues, all administered glucose not utilized is excreted promptly in the urine.

2. Evidence is presented which indicates that insulin results in a transient increase in sugar combustion and a more prolonged augmentation of the rate of glycogen storage, and that epinephrin causes a net loss of glycogen from the body.

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THE USE OF CONSTANT GLUCOSE INJECTIONS FOR THE STUDY OF INDUCED VARIATIONS IN CARBOHYDRATE METABOLISM

IV. Suppression of Glucose Combustion by Continuous Prolonged Epinephrin Administration

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Most of the effects of epinephrin on carbohydrate metabolism have been well established by frequent repetition. It is now conceded generally that it causes hyperglycemia, glycosuria, and mobilization of glycogen and that it affords prompt relief for symptoms of insulin overdosage, but its effect on carbohydrate oxidation is still somewhat uncertain. As noted by Lusk (1), the effect of a single dose appears to depend upon the nutritional state of the subject, a conception which is substantiated by the recent findings of Cori and Cori (2) under carefully controlled feeding conditions. The immediate increase in the rate of metabolism appears to take place at the expense of the fuel most readily available. The problem is complicated by the fact that the elimination of carbon dioxide may be increased by processes of a non-metabolic nature (excess lactic acid production and over-ventilation (3)), and the immediate respiratory data are subject to some doubt on this account.

Most investigators agree, nevertheless, that combustion of carbohydrates, if available, may be increased after epinephrin administration, or at least that the drug exerts no specific influence in the opposite direction. The only contrary findings appearing in the literature are those of Wilenko, (4) who found that whereas glucose given alone always produced typical increases in the respiratory quotient of 12 to 31 per cent, when epinephrin was administered simultaneously the greatest increase observed was 8.8 per cent, and often no change whatsoever occurred.

The present experiments show that prolonged epinephrin administration suppresses glucose oxidation completely. This probably explains, in part, why different investigators using different doses obtain inconsistent results. Given subcutaneously, the effect of a single dose upon glucose

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combustion would be determined by the duration of its influence and hence by the size of the dose and the rate of its absorption. The ability of epinephrin to suppress oxidation also appears to be capable of explaining certain facts, which will be discussed later, in connection with the pathogenesis of diabetes mellitus.

Most of the methods employed have been described previously in detail (5). Briefly reviewed, they consist of continuous estimations of the rates of glucose oxidation and excretion in female cats uniformly anesthetized by means of amytal and receiving glucose by vein at constant rates. It has been shown that normally under such conditions the rate

TABLE 1

Suppression of glucose oxidation by continuous administration of epinephrin, in spite of excessive glucose supply (see chart 1)

Experiment 118: Cat 325, weight 2.64 kgm. Diet exclusively milk and lactose for 3 days previously.

Glucose supply 10.8 cc. 10.3 per cent solution or 1110 mgm. per hour = 0.42 gram per kgm. per hour.

Epinephrin supply: 0.16 mgm. per hour = 0.001 mgm. per kgm. per minute.

HOUR	BLOOD SUGAR*	URINE NITR.	RESPIRATORY EXCHANGE						SUGAR EXCRETED
			CO_2		O_2		R.Q.		Sugar oxidized
			Total	N.P.	Total	N.P.	Total	N.P.	
	per cent	*mgm.*	*cc.*	*cc.*	*cc.*	*cc.*			*mgm.*	*mgm.*
0–1	0.305	61.3	922	626	1074	704	0.859	0.889	586	599
1–2	0.313	52.1	927	675	1121	806	0.827	0.837	480	1200
2–3	0.333	59.4	928	641	1206	847	0.770	0.756	190	1640
3–4	0.336	64.1	934	624	1225	838	0.763	0.745	144	1820
4–5	0.329	66.5	934	612	1231	830	0.758	0.738	114	1910
5–6	0.317	63.3	892	585	1208	826	0.738	0.709	6	1740
6–7	0.320	60.2	893	602	1218	854	0.733	0.705	0	1570
7–8	0.333	56.8	902	627	1243	900	0.725	0.696	0	1440

* Taken at end of period.

of oxidation invariably increases during the first few hours of the injection and that ultimately it and the glycosuria together account for a constant proportion, usually 70 to 90 per cent, of the supply (5).

In the present experiments all procedures were the same as those described previously except that in addition epinephrin (Parke, Davis & Co.'s adrenalin, 1–1000) was mixed with the glucose solutions and injected intravenously with them at constant rates. The concentration of epinephrin was adjusted in each solution so that the required rate of glucose administration would result in the injection of epinephrin simultaneously at a rate of 0.001 mgm. per kgm. per minute, or 0.06 mgm. per kgm. per hour. This dosage was selected arbitrarily on the basis of the quanti-

tative assays of Cannon and Rapport (6) and Kodama (7), which indicate that during reflex stimulation of the adrenals the secretion of epinephrin may proceed at rates as great as about 0.005 mgm. per kgm. per minute, or five times the dose selected. Thus the quantities administered appear to lie well within maximal physiological limits, although above the estimated common rate of secretion under experimental conditions.

RESULTS. The findings in experiments 118, 119, and 123, shown graphically in charts 1, 2, and 3 and tabulated in tables 1, 2, and 3, require little elaboration. They demonstrate clearly that when epinephrin is given *continuously* in amounts within maximal physiological limits the response to injected glucose is altered as follows.

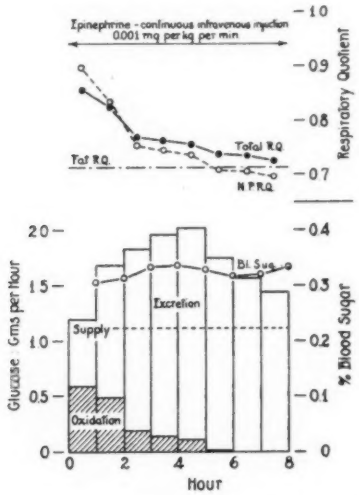

Chart 1. Experiment 118. See table 1

1. Instead of rising progressively as under identical conditions without epinephrin, the non-protein respiratory quotient declines to the fat level within 3 to 5 hours, indicating no glucose oxidation, and remains there as long as the epinephrin administration persists.

The low values for both quotients observed in the second and third hours of experiment 119 (chart 3, table 3) are explicable on the basis of over-ventilation during the first hour and subsequent retention of carbon dioxide. It may be noted that in all three experiments the initial values are higher than the corresponding ones obtained in control experiments without epinephrin (reported previously (5)). This is probably due to non-metabolic factors induced at the outset by the epinephrin, and

therefore the significance of the abrupt decline during the first hour or two might be questioned. It is also true, nevertheless, that such factors could not explain the subsequent failure of the carbon-dioxide elimination to rise.

2. Withdrawing the epinephrin permits the non-protein respiratory quotient and calculated glucose oxidation to rise promptly and progressively (expt. 119, table 3, chart 3). This observation serves as a valuable control for the conditions employed.

TABLE 2

Suppression of glucose oxidation by continuous epinephrin supply, in spite of continuous glucose supply (see chart 2)

Experiment 123: Cat 329, weight 2.45 kgm. Deprived of food 72 hours previously.

Glucose supply: 12.6 cc. 5.00 per cent solution or 628 mgm. per hour = 0.26 gram per kgm. per hour.

Epinephrin supply: 0.15 mgm. per hour or 0.001 mgm. per kgm. per minute.

HOUR	BLOOD SUGAR*	URINE NITR.**	RESPIRATORY EXCHANGE							SUGAR EXCRETED
			CO_2		O_2		R.Q.		Sugar oxidized	
			Total	N.P.	Total	N.P.	Total	N.P.		
	per cent	*mgm.*	*cc.*	*cc.*	*cc.*	*cc.*			*mgm.*	*mgm.*
0	0.078									
0–1	0.212	53.5	915	515	1064	565	0.860	0.913	531	1
1–2	0.278	109.0	971	571	1243	744	0.781	0.768	206	377
2–3	0.323	85.7	918	518	1229	730	0.747	0.710	8	754
3–4	0.329	79.5	873	488	1191	711	0.733	0.687	0	1160
4–5	0.317	65.5	819	502	1153	757	0.710	0.663	0	1100
5–6	0.325	53.5	821	562	1136	813	0.723	0.691	0	879
6–7	0.327	43.1	829	620	1109	849	0.747	0.731	91	681
7–8	0.333	39.5	776	585	1065	826	0.729	0.709	4	690
8–9	0.329	37.8	792	609	1090	861	0.727	0.707	0	645
9–11	0.313	37.5‡	—	—	—	—	—	—	—	606‡

* Taken at end of period.

** Average nitrogen for first three hours used in calculations for those periods because of obvious washing out in the second.

‡ Per hour.

3. Whereas ordinarily (without epinephrin) the glucose oxidation and excretion together account eventually for a constant proportion of the glucose supply, usually 70 to 90 per cent, under the influence of epinephrin they account initially for a variable quantity of extra glucose, presumably from glycogen. Later, when oxidation is abolished, the excretion tends to equal the supply in all cases. In two experiments (123 and 119) this total excretion of the supply occurred with rates of glucose administration which ordinarily (without epinephrin) cause no abnormal glycosuria whatsoever.

The brevity of this balanced phase in which all of the injected glucose appears in the urine, as well as the fact that the excretion tends to decline very slowly, make the assumption of a continuous total recovery of the injected glucose somewhat dubious. It is difficult to settle this point because more prolonged observations are impracticable if not technically impossible with the experimental conditions employed. It is worth noting, however, that in the two longest experiments the excretions showed a distinct tendency to form a plateau at the supply level during the last few hours.

It would appear, therefore, that epinephrin administered *continuously* by vein, at a rate well below that of maximal physiological secretion,

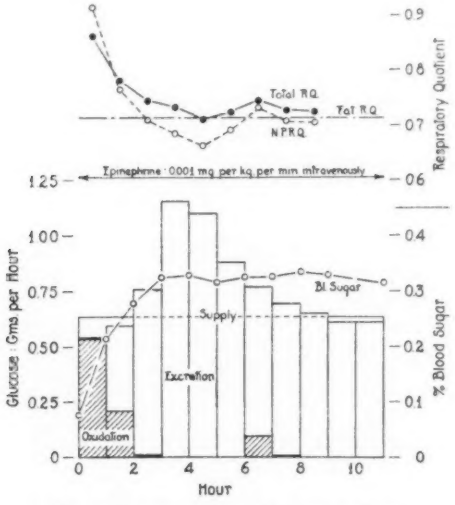

Chart 2. Experiment 123. See table 2

discharges glycogen, suppresses glucose combustion even in the presence of an excessive supply, and may in fact completely abolish the ability to utilize glucose in any manner, as indicated by the ultimate total excretion of the supply. Obviously this combination of consequences is identical with that existing in total diabetes, insofar as glucose metabolism is concerned.

The analogy of this condition induced by epinephrin with that present in total diabetes may be carried further by an analysis of the findings in the last nine hours of experiment 119 (table 3, chart 3). During the last five hours of epinephrin administration (6th to 10th inclusive) the non-protein respiratory quotient was maintained at or near the fat level, the blood

sugar concentration was high, there was heavy glycosuria equal to the supply, indicating no glycogen storage, and the protein metabolism was fairly constant. Withdrawing the epinephrin resulted in an increased respiratory quotient and hence increased glucose oxidation, in a markedly reduced glycosuria, hyperglycemia, and protein metabolism, and in an

TABLE 3

Suppression of glucose oxidation by continuous epinephrin administration, in spite of continuous glucose supply. Subsequent recovery of oxidation upon withdrawing epinephrin (see chart 3)

Experiment 119: Cat 326, weight 2.32 kgm. Diet milk and lactose only for 2 days previously.

Glucose supply: 15.1 cc. 5.00 per cent solution or 760 mgm. per hour = 0.33 gram per kgm. per hour.

Epinephrin supply: 0.15 mgm. per hour = 0.001 mgm. per kgm. per minute for first 10 hours only.

HOUR	BLOOD SUGAR*	URINE NITR.	RESPIRATORY EXCHANGE						SUGAR OXIDIZED	SUGAR EXCRETED	REMARKS
			CO_2		O_2		R.Q.				
			Total	N.P.	Total	N.P.	Total	N.P.			
	per cent	*mgm.*	*cc.*	*cc.*	*cc.*	*cc.*			*mgm.*	*mgm.*	
0–1	0.279	73.9	796	439	956	510	0.833	0.861	357	372	Epinephrin continuously
1–2	0.329	70.0	744	406	991	568	0.751	0.714	16	666	by vein at rate of 0.001
2–3	0.343	65.8	745	427	968	570	0.770	0.749	109	690	mgm. per kgm. per min.
3–4	0.350	63.8	760	451	984	599	0.772	0.753	126	704	Glucose at rate of 0.33
4–5	0.355	62.7	768	464	999	620	0.769	0.749	118	758	gm. per kgm. per hour
5–6	0.360	56.6	712	438	963	621	0.739	0.705	0	766	
6–7	0.357	54.9	707	441	972	640	0.728	0.690	0	780	
7–8	0.360	52.6	Lost**	—	—	—	—	—	—	766	
8–9	0.363	49.8	683	442	921	621	0.741	0.712	12	734	
9–10	0.360	47.3	712	483	973	687	0.732	0.703	0	727	
10–11	0.347	33.3	677	516	916	715	0.739	0.722	47	468	Epinephrin stopped.
11–12	0.327	23.5	620	506	835	693	0.743	0.731	75	237	Glucose continued
12–13	0.294	21.0	630	529	825	698	0.764	0.757	159	140	
13–14	0.238	19.9	643	546	828	708	0.776	0.772	209	64	

* Taken at end of period.

** Overflow from spirometer during collection. Results markedly discrepant with those of surrounding periods so were discarded.

accumulation of glycogen (glucose supplied but unaccounted for). This is exactly what happens in total diabetes under analogous conditions after the administration of insulin.

The exact mechanism responsible for the observed alterations in glucose metabolism induced by epinephrin cannot be deduced from the data at hand. Several possibilities must be considered. Hypothetically the ad-

ministered glucose may have been "inactivated" by the epinephrin *in vitro* before injection, in some manner which changed it to an unutilizable form. The writers know of no evidence for this view. In this case glucose combustion should have proceeded during the administration of the "epinephrinized glucose" as it does in fasting, i.e., some oxidation of preformed glycogen might reasonably be expected to have continued. The complete cessation of glucose combustion makes this view improbable.

The second possibility is suggested by the findings of Cori and Cori (2) which indicate that epinephrin accelerates glycogen mobilization and in-

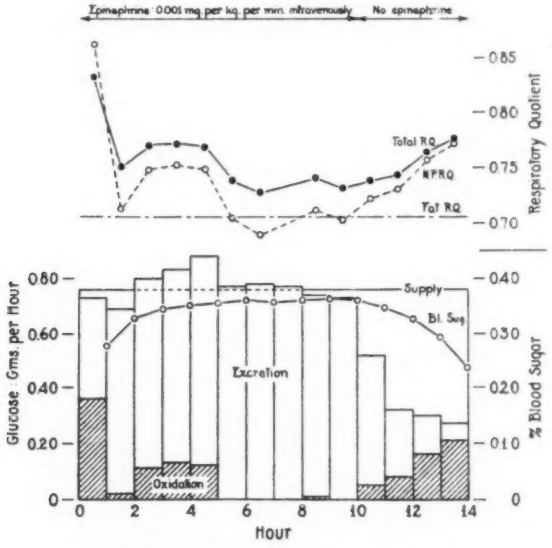

Chart 3. Experiment 119. See table 3

hibits glycogen synthesis everywhere except in the liver. If this is true, continued epinephrin administration might conceivably exhaust the glycogen reserves everywhere except in the liver and ultimately suppress glucose oxidation because none would be available peripherally. This process might occur independently or as a corollary of the third possibility to be mentioned. It was not investigated further.

The phenomenon could also be explained readily if it is assumed that insulin production was suppressed as a result of the continued action of epinephrin, i.e., a virtual pancreatectomy by inhibition of function. The time relations favor this view inasmuch as the effects on glucose combustion of starting and stopping the epinephrin appear gradually over periods

of several hours, just as the effects of removing or increasing the insulin supply do. (See (8), charts 1, 2, 3, 5, 6.)

This latter possibility has been investigated further with results which are, on the whole, non-decisive. If continued epinephrin administration suppresses insulin production, its administration into a fasting animal should accomplish, as far as known, the same results as surgical removal of the pancreas, namely, continuous glycosuria, eventually from protein as indicated by ultimate proportional excretions of glucose and nitrogen.

Six attempts were made to demonstrate this phenomenon. Epinephrin

TABLE 4

Effect of continuous epinephrin administration during fasting (see chart 4)

Experiment 126: Cat 332, weight 2.30 kgm. Deprived of food 72 hours previously. Epinephrin administration: 6.9 cc. per hour of solution containing 2.0 mgm. epinephrin per 100 cc. = 0.138 mgm. per hour or 0.001 mgm. per kgm. per minute, given intravenously. Signs of circulatory failure after 19th hour. Cat died 6 hours after end of experiment.

Values for glucose and nitrogen are superimposed in chart.

HOUR	BLOOD SUGAR*	URINE		
		Glucose	Nitrogen	D/N
	per cent	*mgm.*	*mgm.*	
0–11	0.225	277**	64.6**	4.29
11–12	0.226	142	50.4	2.82
12–13	0.221	94.8	45.4	2.09
13–14	0.215	55.5	51.8	1.07
14–15	0.206	50.7	44.8	1.13
15–16	0.200	45.5	44.8	1.02
16–17	0.201	50.0	48.3	1.04
17–18	0.201	56.5	48.4	1.17
18–19	0.199	44.3	44.2	1.00
19–21	0.182	28.6**	37.0**	0.77

* Taken at end of period.

** Average per hour.

alone was administered intravenously at a rate of 0.001 mgm. per kgm. per minute continuously for periods of 21 to 48 hours into amytalized cats which had fasted 2 to 7 days previously. In all but one case gross glycosuria appeared promptly, reached a maximum within the first few hours, and then declined steadily until it vanished after 15 to 23 hours. The blood-sugar concentration followed an approximately parallel course but tended to continue at a slightly increased level (0.16 to 0.20 per cent) after the glycosuria had subsided. Excretion of nitrogen persisted unchanged in all cases after abnormal glycosuria had ceased.

In only one case was there any indication of excretion of protein sugar

(expt. 126, chart 4, table 4). Here after the 13th hour the glycosuria and hyperglycemia became constant for six successive hourly periods. During this time the nitrogen excretion also was constant and the D to N ratio was maintained evenly at a level of 1.1. The fact that all values fell off perceptibly after the 19th hour cannot be considered seriously since the animal began simultaneously to show signs of circulatory failure and died six hours later.

Attempts to recover single doses of glucose injected at the height of the epinephrin influence, i.e., after the initial glycosuria had subsided, have likewise been disappointing. In one case about 400 of the 500 mgm. in-

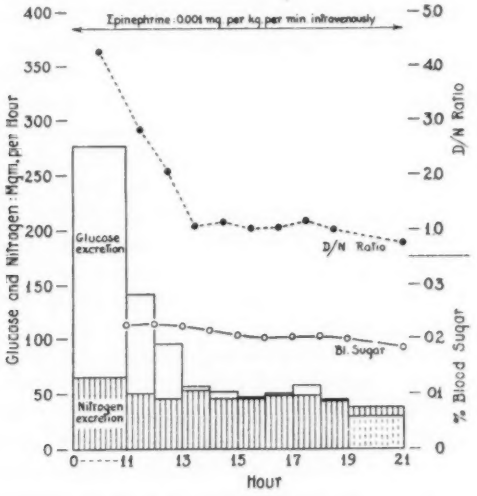

Chart 4. Experiment 126. See table 4. Values for glucose and nitrogen are superimposed.

jected appeared in the urine within 4 hours, but four other attempts resulted in recoveries of 25 per cent or less.

Thus, efforts to reproduce the picture caused by pancreatectomy have been inconclusive. Yet in certain respects they are suggestive. Several possibilities which might explain this failure to obtain consistent results suggest themselves immediately. In the first place there is no reason to believe that the dosage of epinephrin arbitrarily selected is ideal for the purpose. Secondly, experience with this particular experimental subject forces the conclusion that reliable data cannot be obtained consistently for periods longer than 20 hours or so. As stated previously, anesthesia may be maintained uniformly with ease for shorter periods in the manner out-

lined, but when more prolonged observations are required a different subject would be desirable.

Discussion. The existing belief concerning the effect of epinephrin on glucose oxidation is based on observations following a single dose subcutaneously, as a rule, or at best repeated doses given at variable intervals. It scarcely seems necessary to point out that unless the drug is given at short intervals for considerable periods of time a sustained epinephrin excess without lapses could not be expected. This probably explains the fact that suppression of glucose oxidation after epinephrin has escaped attention previously. Perfusion experiments with epinephrin like those of Patterson and Starling (9) in heart lung preparations ordinarily are closed after three or four hours at most and therefore are not comparable.

Nevertheless the observations reported herein should be confirmed under different conditions if possible. The necessity of employing anesthesia will undoubtedly cast some doubt upon the applicability of the findings in normal physiology, even though it has been demonstrated repeatedly that uniformly amytalized animals respond to administered glucose in a manner qualitatively identical with that of normal animals (5), (8).

Assuming for the moment, however, that the interference with glucose oxidation established above is applicable in normal physiology, it is conceivable that the etiology of diabetes mellitus is related to the fact. It may be pertinent in this connection to review certain other evidence bearing on the point in question.

The idea of a nervous basis for diabetes mellitus, originating with Claude Bernard, was studied extensively during the fifteen or twenty years following the discovery of the effects of pancreatectomy. This older work, reviewed thoroughly by Allen (10), contributes little to an understanding of the pathogenesis of diabetes except proof that the nerve injury associated with surgical removal of the pancreas is not of itself sufficient to produce the disease, and that the pancreas may function properly without its original nerve supply.

In spite of the negative character of this older work the idea that diabetes mellitus is due to a functional deficiency of the pancreas persists and is even strengthened by accumulating information. Of outstanding importance is the fact that it is often impossible to demonstrate sufficient pathological change in the pancreas of a diabetic individual to account for the associated diabetes, if indeed for any diabetes whatsoever. Specific examples of this fact, recognized for some time, may be found in recent case reports by Warren (11). Woodyatt, discussing the discrepancy and its significant possibilities, points out that"—the symptomatology of ordinary diabetes and the morbid anatomic findings or absence of findings suggest a disease of the sympathetic autonomic nervous system" (12).

Independent observations supporting this viewpoint are not lacking.

The frequent onset of diabetes, or loss of tolerance when already established, during emotional stress is a common clinical incident (13). Brugsch, Dresel and Lewy (14) found retrograde degenerative changes in some of the brain stem nuclei after pancreatectomy as well as in cases of human diabetes. Urechia and Nitescu (15) have confirmed the former observation in dogs and Urechia and Elekes (16) and Nicolesco and Răileanu (17) the latter in man. Many examples of changes in the coeliac plexus are also quoted by Urechia and Nitescu. The recent monograph by Leschke (18) includes a comprehensive review of the subject.

Permanent diabetes mellitus has never been produced in the normal animal by injury of the nervous system, but Bernard observed glycosuria lasting for days after medullar puncture (19). Camus, Gournay and Le Grand (20) noted heavy glycosuria lasting for several weeks in rabbits after implantation of irritating foreign bodies into the hypothalamus, and Allen (21) has been able to transform mild diabetes under dietary control into a severe and fatal form with acidosis by performing medullar punctures on partially depancreatized dogs. Thiroloix (22) demonstrated a similar phenomenon earlier.

There are in addition innumerable illustrations of transient hyperglycemia and glycosuria following nervous or emotional disturbances of one type or another. These are ordinarily explained on the basis of glycogen mobilization alone. The experimental evidence just cited, as well as that reported in the present paper, indicates that some other influence must be involved, specifically a temporary depression of the total power of sugar utilization, including oxidation as well as storage, possibly due to a temporary depression of insulin production. The possibility of reproducing permanent diabetes experimentally in this manner would naturally be limited by technical difficulties.

In brief, therefore, the following related facts are apparent. Independent nervous lesions may be found in diabetes and distinctive pathology of the pancreas is often lacking. Similar lesions may produce symptoms inexplicable on any other basis than depression of total sugar utilizing ability, possibly by interfering with insulin production. The investigations conducted by Cannon and his collaborators within the past ten years prove beyond doubt that epinephrin secretion is augmented as a result of various disturbances involving the central nervous system reflexly or directly. Continuous physiological excesses of epinephrin secretion on this basis are not inconceivable. The position of the present findings in this scheme is not entirely clear. Before any final conclusion can be made it must be established that continuous adrenal over-activity, alone or in conjunction with sympathetic nervous influences, may depress insulin secretion. The fact that continuous epinephrin administration may suppress glucose combustion and polymerization provides strong support for such

an assumption, and hence for the view that the insulin deficiency responsible for diabetes mellitus is due primarily to a disorder remote from the pancreas, probably, as suggested by Woodyatt, to a disease of the sympathetic nervous system.

It will be recalled that Zuelzer believed that diabetes mellitus was due to excessive activity of the adrenals (23). His idea was based on evidence which later was confirmed by others (24), but which has been questioned by Stewart and Rogoff (25). It is unnecessary to consider this questionable evidence because the picture is complete without it.

SUMMARY

1. Glucose oxidation is abolished by prolonged administration of epinephrin intravenously at a rate of 0.001 mgm. per kgm. per minute. This occurs in spite of a constant intravenous supply of glucose. Since glucose oxidation invariably occurs under identical conditions without epinephrin, the use of anesthesia in the present investigations is in no way responsible for the phenomenon.

2. Attempts to reproduce the results of pancreas removal in this manner have been inconclusive. It is not clear, therefore, whether the observed effect of epinephrin is due to a suppression of insulin production or not.

3. Contemporary evidence favors the view that ordinary diabetes mellitus is a result of a functional disorder of the pancreas which is dependent upon a disease of the sympathetic nervous system, and that continuous excessive secretion of epinephrin may be an important intermediate factor in this mechanism.

We wish to acknowledge a debt of gratitude to Prof. Walter B. Cannon for his aid in the conduct of these investigations.

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THE EFFECT OF THE DAILY ADMINISTRATION OF IODINE ON THE CALORIGENIC ACTION OF SINGLE INTRAVENOUS INJECTIONS OF THYROXINE[1]

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The striking effect of iodine in lowering the surgical mortality in cases of exophthalmic goiter, as shown by Plummer, is usually accompanied by lowering of the basal metabolic rate. Recent studies by Gray and Loeb, and Rabinovitch have shown that the administration of iodine to normal guinea pigs caused marked changes in the microscopic anatomy of the thyroid gland. These observations naturally raise the significant question of whether iodine will influence the response of normal animals to a single intravenous injection of thyroxine. In an investigation of this kind it is essential to employ a dose of thyroxine which will produce constant, definite, but not excessive, elevation of the metabolic rate without severe reactions such as excessive loss of weight, diarrhea and vomiting, since, if these phenomena are present, they may introduce secondary and variable factors which may influence the results. The quantity of thyroxine which will be necessary to produce this desired reaction will undoubtedly vary in different animals.

As will be mentioned, several investigators have shown clearly that the administration of iodine, either in the form of the compound solution of iodine (Lugol's solution) or as potassium iodide, will not prevent the elevation of metabolism which follows the feeding or injection of thyroid extract or thyroxine, nor does it hasten the return of the metabolism to normal after thyroid feeding has been discontinued. The present study is of a somewhat different nature and is primarily concerned with a quantitative study of the effect of iodine on the response of a normal animal to a single intravenous injection of thyroxine.

METHODS. The experiments reported here were performed on a dog which had been used in studies of metabolism for about two years; it was perfectly trained and thoroughly accustomed to the various experi-

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mental procedures. During the entire period the animal had been receiving a constant amount of the standard metabolism diet employed in the laboratory, and the weight and basal metabolism had remained practically constant; during the four months preceding the experiments the maximal variation in weight had been from 7.4 to 7.5 kgm.; the basal level of heat production and basal respiratory quotients (twenty-one hours after the last feeding) had likewise shown the usual constancy, the average maximal variation in the former amounting to 1.1 calories for each hour (from 10.7 to 11.8 calories) with an average of 11.1 calories, while the average respiratory quotient had varied between 0.75 and 0.80, with an average of 0.77. Attention should be called to the fact that bilateral oöphorectomy had been performed on this animal about two years previously. This procedure is carried out on many of our metabolism dogs because we have noted that during estrum the standard metabolism is likely to be somewhat irregular; oöphrectomy eliminates this complicating factor in experiments in which we wish to obtain strictly comparable data over relatively long periods.

The dose of thyroxine employed in all experiments was 10 mgm. This amount was dissolved in about 5 cc. of distilled water to which was added 1 drop of 10 per cent sodium hydroxide and was injected intravenously, care being taken to empty the syringe by withdrawing and reinjecting blood several times. The respiratory metabolism was determined at regular intervals following the injection of thyroxine and the entire curve representing the elevation and return of the heat production to normal was obtained. During the entire experimental period the animal received the usual quantity of standard diet at approximately the same time each day, the metabolism tests being performed twenty-one hours after the last feeding.

The respiratory metabolism was determined by the method of Boothby and Sandiford which was adapted to animal use by Kitchen. The analyses of expired air were always made in duplicate and a test was not considered satisfactory unless the duplicate analyses agreed within 0.03 per cent for carbon dioxide and 0.04 per cent for oxygen. The environmental temperature was maintained approximately constant between 25° and 30°C. Use was not made of the results of any test in which there was the slightest evidence of restlessness on the part of the animal. A series of from thirteen to fifteen tests, covering a period of approximately four hours, was carried out on each experimental day and the average was used to represent the metabolism for that day.

It will be necessary to mention briefly the manner in which certain secondary calculations were made from the data so obtained. The average figure for calories of heat production each hour, oxygen consumption and carbon dioxide production in liters per hour, were plotted on coördinate paper, as shown in the illustration. By means of a standardized planim-

eter the area between the curve after injection of thyroxine and the corresponding base line was determined and converted into calories or liters as the case might be. These values represented the extra calories of heat production, liters of oxygen consumption and carbon dioxide production following the injection of thyroxine. The total oxygen consumption and carbon dioxide production for the entire period following the injection of thyroxine were calculated by adding the respective basal values for the entire period to the extra values. From the total oxygen consumption and total carbon dioxide production we have calculated the total heat produc-

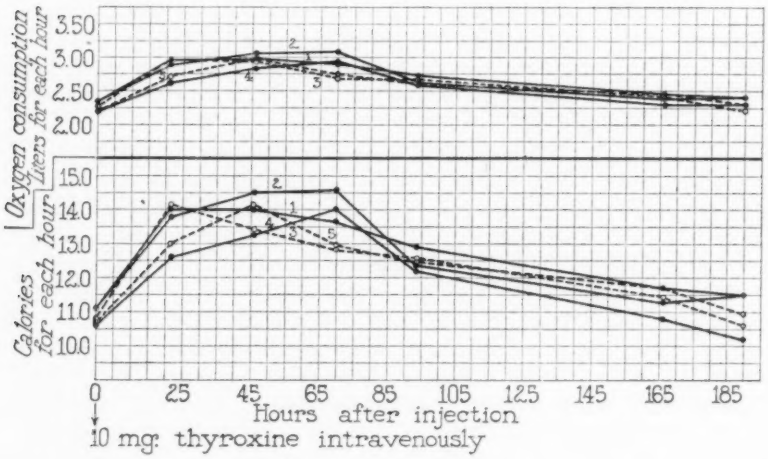

Fig. 1. Curves of the oxygen consumption in liters per hour and the heat production in calories for each hour during the period of one hundred ninety hours after the intravenous injection of 10 mgm. of thyroxine, in three experiments without iodine (solid lines), and in two experiments in which iodine was administered for eighteen and twenty-two days before and for eight days after thyroxine (dotted lines).

tion and respiratory quotients for the entire period of eight days after injection of thyroxine.

A series of five experiments was performed on the same dog. In three of the experiments 10 mgm. of thyroxine were given without the coincident administration of iodine, and in the remaining two experiments iodine was administered before and after the injection of thyroxine. Iodine was administered in the form of the compound solution of iodine and the dose employed was 2 cc. daily; this quantity was added to about 100 cc. of water and administered by stomach tube. In one experiment iodine was administered daily for eighteen days before and eight days after the injection of thyroxine, while in the second it was given for twenty-two days

before and eight days after the thyroxine. Untoward symptoms which could be ascribed to the iodine were not noted, except slight coryza which developed during the latter part of the first experiment.

RESULTS. The animal remained in perfect health during the entire experimental period; the loss of weight was slight and the dose of thyroxine employed did not cause nausea, vomiting or diarrhea, reactions which may occur in severe cases of experimental hyperthyroidism produced by repeated daily injections of thyroxine (table 1).

The illustration shows quite clearly that the daily administration of iodine does not cause significant changes in the nature of the response to the intravenous injection of 10 mgm. of thyroxine. Following the intravenous injection of 10 mgm. of thyroxine, the heat production rises rapidly and may reach a maximum in from twenty-two to seventy hours, following

TABLE 1

Results of experiments with and without iodine after administration of thyroxine

EXPERIMENT	EXTRA CALORIES PRODUCED DURING 190 HOURS AFTER THYROXINE WAS ADMINISTERED	EXTRA OXYGEN CONSUMED DURING 190 HOURS AFTER THYROXINE	TOTAL CALORIES PRODUCED DURING 190 HOURS AFTER THYROXINE	TOTAL OXYGEN CONSUMED DURING 190 HOURS AFTER THYROXINE	TOTAL CARBON DIOXIDE PRODUCED DURING 190 HOURS AFTER THYROXINE	RESPIRATORY QUOTIENT OF METABOLISM FOR A PERIOD OF 190 HOURS AFTER THYROXINE
		liters		*liters*	*liters*	
Without iodine	312	73	2,432	516	375	0.73
Without iodine	307	70	2,416	513	374	0.73
With iodine	299	72	2,355	500	362	0.73
Without iodine	281	70	2,330	491	371	0.76
With iodine	323	79	2,366	499	377	0.75

which there is a gradual decline. The entire curve representing the rise and subsequent return to normal requires approximately eight days (one hundred ninety hours). In the three control experiments, in which iodine was not administered, the extra heat production during the period of one hundred ninety hours amounted to 312, 307, and 281 calories respectively, with an average of 300 calories. In the two experiments in which iodine was administered before and after the thyroxine, the extra heat production for the same period amounted to 299 and 323 calories respectively, with an average of 311 calories. The maximal variation between the high value of 323 calories occurring in the second experiment in which iodine was administered, and the low value of 281 which was obtained in the third experiment without iodine, amounted to approximately 15 per cent. The average extra heat production for the five experiments was 304 calories, and the maximal and minimal values were within $\pm$ 8 per cent of this

average. The average extra oxygen consumption during the total period of one hundred ninety hours for the five experiments amounted to 73 liters, with maximal and minimal values of 70 and 79 liters respectively, which are again within ±8 per cent of the average.

In considering the total heat production during the period of one hundred ninety hours after thyroxine was given, the same constancy was found. The average for the five experiments was 2380 calories. The maximal value of 2432 calories and the minimal value of 2330 calories occurred in the control experiments without iodine and was within ±2 per cent of the average. The respiratory quotients calculated from the total oxygen consumption and carbon dioxide production for the period of one hundred ninety hours after thyroxine had been given were likewise seen to be constant, varying from 0.73 to 0.76, and apparently were not influenced by the administration of iodine.

COMMENT. These experiments show clearly that the daily administration of iodine over short periods does not appreciably influence the response to a single intravenous injection of 10 mgm. of thyroxine. In clinical cases of exophthalmic goiter the effect of iodine usually becomes manifest on about the seventh to the tenth days by a drop in the basal metabolic rate. Rabinovitch recently showed that the stimulating effect of potassium iodide on the mitotic activity of the thyroid gland in normal guinea pigs reaches its maximum on about the fifteenth to the sixteenth days; in view of these two observations it seems likely that in our experiments iodine was administered over a sufficiently long period to have brought about any possible effect on the calorigenic action of thyroxine.

The daily administration of iodine, in the doses employed, did not appreciably influence the basal level of heat production. In the first experiment there was a slight reduction which averaged approximately 0.3 calorie per hour, but since this was accompanied by slight loss of weight it was probably not a specific effect of the iodine; in the second experiment the iodine did not produce a change in the basal heat production. Marine and Lenhart, and Webster and Chesney noted that the administration of iodine to dogs and rabbits with hyperplastic thyroid glands caused rapid loss in weight, but that this did not occur in normal animals. The thyroid gland of the animal used in these experiments was not palpably enlarged. Several other investigators (Marine et al., Snell et al., Webster and Chesney) have noted irregular and in general rather small variations in basal heat production after the administration of iodine to normal human and animal subjects.

Kunde showed that in dogs the administration of iodine will not reduce or prevent the elevation in heat production which results from the feeding of thyroid extract or repeated injections of thyroxine. Sturgis and his co-workers, employing rabbits, showed that the same animals responded in

the same manner to three successive daily doses of 1 mgm. of thyroxin given with and without the concurrent administration of iodine. Cordonnier demonstrated that in guinea pigs the administration of potassium iodide does not hasten the return of the heat production to normal after thyroid feeding had been discontinued.

SUMMARY

The daily oral administration of iodine for seventeen and twenty-two days before and for eight days after a single intravenous injection of 10 mg. of thyroxine did not influence the calorigenic action of thyroxine. Following the injection of thyroxine the basal heat production rose rapidly and reached its maximal height in from twenty-two to seventy hours; following this there was a progressive decrease in heat production which reached the original basal level in about one hundred ninety hours (eight days). In three control experiments in which iodine was not given the extra heat production averaged 300 calories, and in two experiments in which iodine was given the average was 311 calories.

The response of the same animal to repeated single injections of 10 mgm. of thyroxine was constant; the average extra heat production in five experiments amounted to 304 calories, and the maximal and minimal values obtained were within ±8 per cent of this average.

The daily oral administration of iodine (2 cc. of the compound solution of iodine) was without appreciable influence on the basal level of heat production.

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RELATION OF THE FOLLICULAR AND CORPUS LUTEUM HORMONES IN THE PRODUCTION OF PROGESTATIONAL PROLIFERATION OF THE RABBIT'S UTERUS[1]

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Since the beginning of the twentieth century many studies have been made to determine the function of the corpus luteum of the ovary by means of extracts. Some of the physiological tests used for determining the potency of these various preparations have been inhibition of ovulation, growth and changes in the reproductive tract, lactation, growth of the mammary glands, and relaxation of the pelvic ligaments of the guinea pig. These tests have been applied chiefly to the various species of laboratory rodents, although birds and monkeys have been used. With the preparation in this laboratory of a water soluble extract of the corpus luteum of the sow which is capable of producing many of these physiological changes in the female reproductive tract of the rat, mouse and guinea pig, it is the object of this paper to present an account of the effect of this preparation on the uterus of the rabbit.

Bouin and Ancel (1909) showed the normal relationship of the corpus luteum to the peculiar changes in the uterine endometrium of the rabbit for the reception and early development of the young. This has been confirmed many times by various workers such as Hammond (1925) and more recently Joublot (1928) and Corner (1928). The endometrium of the corpus luteum shows distinct characteristics which cannot be confused with any other change that might be brought about normally by the ovary or by ovarian extracts. The uterus is large, the glands are greatly enlarged and deeply embedded in the sub-mucosa which, when seen in cross section, gives the endometrium a ragged appearance. This condition of the uterus, attributed to functional corpora lutea, may be found in normal pregnant rabbits about five days after copulation or in the pseudopregnant rabbit five days after sterile copulation.

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This typical uterine picture is spoken of as a pseudopregnant uterus or progestational proliferation of the uterine endometrium and can be readily distinguished from a uterine picture of full oestrum. At the time of full oestrum, the uterus is large, with a thick endometrium but the uterine glands are not noticeably dilated. The epithelial lining is smooth and not typically ragged as in the other case when the uterus is subjected to the action of the corpus luteum.

Before the work of Allen and Doisy (1923) the function of the ovary correlated with anatomical changes in the reproductive tract were rather vague and especially so when extracts were used. As a result, the extracts of the corpus luteum prepared by earlier workers did not seem to be consistent in producing changes in the endometrium of the rabbit uterus that were comparable with the normal. Extracts of the corpora lutea prepared by Iscovesco (1912) prevented involution of the uterus of the rabbit after castration. No plates were given and it is hard to say whether or not he was working with the oestrus producing hormone or the corpus luteum hormone. The methods of extraction seem to indicate that he may have had considerable oestrus hormone present.

Fellner (1913) appears to have been working with the oestrus hormone also for his plates show the typical condition of the uterus of the rabbit just after it has been bred. Seitz, Wintz, and Fingerhut (1914) showed the gross enlargement of the uterus of a rabbit with extracts of corpora lutea. This would indicate the presence of follicular hormone in their extract. Herrmann (1915) studied the effects of extracts of the corpus luteum and the placenta made with lipoid solvents. Injecting the material into immature rabbits he obtained a typical uterus in full oestrum and he also obtained for the first time, apparently, a true progestational proliferation of the endometrium of the rabbit's uterus when using adults. Frank and Rosenbloom (1915), in a preliminary report also give results of extracts of the corpus luteum and placenta. Their plates seem to show for the most part the presence of the oestrus hormone except figure 4, which indicates the possibility of some proliferation of the uterine glands.

Courrier and Potvin (1926) injected follicular hormone into castrated female rabbits and obtained growth and congestion of the uterus, a condition that had in many cases been erroneously attributed to the corpus luteum by several investigators. Courrier and Masse (1928) also injected folliculine into castrate rabbits some days after coitus and observed that this alone was not capable of producing the progestational condition of the uterus typical of the normal corpus luteum. Laqueur, Borchardt, and de Jong (1927) with menformin, obtained similar results. This work only shows that the growth of the uterus of the castrate rabbit depends on the follicular hormone or oestrous producing hormone of the ovary, but fails to demonstrate the physiology of the progestational condition.

While a study of the endometrial changes in the uterus of rabbits was in progress in this laboratory, with water soluble extracts of the corpus luteum of the sow, Corner and Allen (1929) published an excellent paper describing the production of progestational proliferation in the uterus of rabbits castrated in oestrum by the injection of fat soluble extracts of the corpus luteum of the sow. The uterine picture of the injected rabbits was like that of the normal pseudopregnant animals. In addition to confirming these results we wish in this paper to go further into the physiological explanation of the relationship between the follicular and corpus luteum hormones in the production of the reaction.

PROCEDURE. As a general rule sexually mature female rabbits were mated with normal bucks, their ovaries removed within the next few hours

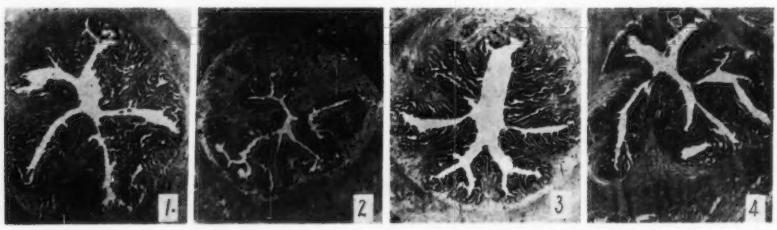

Fig. 1. Uterus of rabbit 5 which was castrated in oestrum and received corpus luteum extracts for the next five succeeding days. Typical progestational proliferation.

Fig. 2. Uterus of rabbit 5 after nine days of corpus luteum treatment. Progestational proliferation not as pronounced as in figure 4.

Fig. 3. Normal pseudopregnant uterus 5 days after copulation.

Fig. 4. Normal pseudopregnant uterus 9 days after copulation.

and a series of injections of corpus luteum extracts started and continued for five to ten days. This procedure was, however, varied to bring out the relationship of the follicular and corpus luteum hormones in the pseudopregnant reaction by injecting each of these substances separately, simultaneously, and successively into mature castrate females. The corpus luteum extract used was the same as that employed to relax the pelvic ligaments of the guinea pig (Hisaw, 1929) and its preparation is given in detail in another paper (Hisaw, Fevold and Meyer, in press). The follicular hormone used in certain of the experiments was kindly furnished by E. R. Squibb & Sons Co., to whom we wish to express our thanks.

EXPERIMENTAL RESULTS. When development of the progestational endometrium of the rabbit is used as a criterion of potency of a corpus luteum extract the most obvious test for its physiological activity is its

ability to replace the normal corpora lutea in pseudopregnancy. This can be carried out by injecting the extract into animals castrated in full oestrum. For example,—rabbit 5 was bred and twenty hours later both ovaries were removed. Ovulation had occurred but no corpora lutea had formed. The rabbit received subcutaneous injections immediately of the corpus luteum extract in 1 cc. doses four times daily, each cubic centimeter being equal to 15 grams of fresh tissue. After five days a portion of one horn of the uterus was removed and sectioned (fig. 1). The corpus luteum injections were then continued for four more days, making a total of nine days in all. The rabbit was then killed and another piece of the uterus sectioned (fig. 2).

A comparison of the five day experimental uterus (fig. 1) with that of a normal five day pseudopregnancy (fig. 3) shows quite clearly that the progestational condition was produced. Likewise a comparison of the nine day experimental (fig. 2) with a nine day pseudopregnant control (fig. 4) demonstrates a positive result. The progestational picture in the nine day experimental, however, does not seem to be quite typical of the pseudopregnant condition of the nine day control. It seems that if the injections of the extract are continued for a longer period than five days the uterus gradually reverts toward the castrate condition even in the presence of the corpus luteum hormone. This gives very suggestive evidence that the corpus luteum hormone though capable of changing the endometrium of oestrum to that of early pregnancy is not able to maintain this condition. It also suggests that some other substance might have been furnished by the ovary of the nine day normal pseudopregnant animal which facilitated the action of the corpus luteum for the preservation of the picture over a longer period.

This opinion was given further support by a study of the effects of corpus luteum extracts on the castrate uterus. For example,—rabbit 3 was bred and eleven hours later both ovaries were removed. After ten days a piece of the uterus was taken out and sectioned, showing a typical castrate condition (fig. 5). Two days were allowed to elapse for recovery from the operation and on the twelfth day injections of corpus luteum extracts were started. This was administered subcutaneously in 1 cc. doses four times daily, each cubic centimeter being equal to 14.5 grams of fresh tissue. After this treatment had been continued for five days the animal was sacrificed, and a sample of the uterus sectioned (fig. 6). The ten day castrate uterus when compared with that after five days of corpus luteum treatment shows very little if any modification. In fact, after the corpus luteum injections the picture is yet that of a castrate animal. These results signify two things, namely, that the corpus luteum hormone alone cannot produce the progestational endometrium in the castrate uterus and that the extract used contained no follicular hormone, or at least not enough to influence the uterus at the dosage used and for the time given.

From the two experiments just cited one might infer that the uterus of the rabbit must be under or recovering from the effects of the follicular hormone before the corpus luteum extracts can produce the progestational changes. We have shown that the corpus luteum extract when given alone has no noticeable influence on the castrate uterus but is it capable of doing so if the castrate animal is first given follicular hormone? That

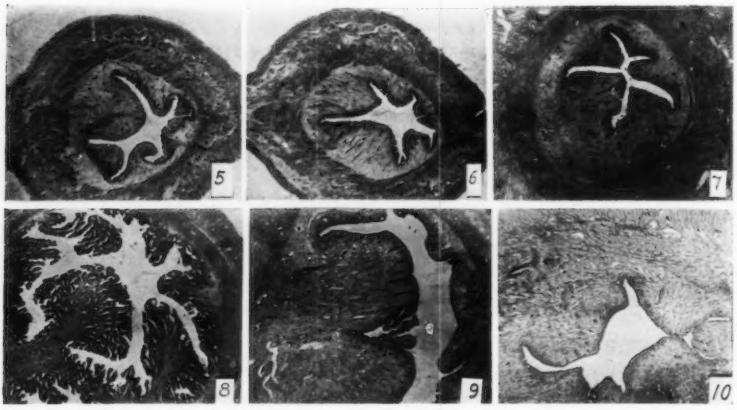

Fig. 5. Section of the uterus of rabbit 3 showing conditions present 10 days after castration in oestrum.

Fig. 6. Section of the uterus of rabbit 3 which was castrated in full oestrum, allowed to wait 12 days after which it received injections of corpus luteum hormone for 5 days. Note that no progestational proliferation has occurred.

Fig. 7. Section of the uterus of rabbit 6 taken at the end of 5 days after castration in full oestrum.

Fig. 8. Rabbit 6. Uterus of a castrate, which was brought into oestrum with follicular hormone and then treated with the corpus luteum hormone for four and one-half days. Typical progestational proliferation.

Fig. 9. Rabbit 7. Uterus of rabbit, castrated in oestrum and treated simultaneously for 5 days with injections of follicular and corpus luteum hormones. Oestrus hormone overrides the corpus luteum hormone.

Fig. 10. Section of uterus showing condition which normally exists at oestrum.

the follicular hormone is important is clearly shown from data obtained from rabbit 6. This animal was mated and five hours later both ovaries were removed. After five days a part of one horn of the uterus was removed and sectioned. At this time the uterus was that of a typical castrate (fig. 7). The animal was then given an oil solution of follicular hormone, 1 cc. twice daily for five days, each cubic centimeter being equal to 20 rat units. It is interesting to note that on the fourth day of this treatment

she received the buck four times. The injections of the follicular hormone were discontinued on the fifth day and the animal was given subcutaneous injections of corpus luteum extracts in 1 cc. doses four times daily for four and one-half days; each cubic centimeter being equal to 13.9 grams of fresh tissue. At the end of this procedure the animal was sacrificed and the uterus removed and sectioned. An examination of figure 8 will show that the characteristic progestational condition of the uterus was produced.

It has already been pointed out that the corpus luteum hormone alone cannot promote progestational growth in the castrate uterus nor can it prolong the condition without the presence of the follicular hormone. A further question that naturally arises then, is that concerning the effects of administering both hormones simultaneously and at the same levels used in the other experiments cited. When a rabbit is castrated in oestrum, is given sufficient follicular hormone to maintain the oestrous condition and at the same time also receives corpus luteum extract in doses large enough to ordinarily produce progestational proliferation the result is not what one may on first thought expect (fig. 9). Under these circumstances the follicular hormone seems to blot out the effects of the corpus luteum hormone and as a result the uterus remains in an oestrous condition. That is, when the corpus luteum extract alone is given immediately after castration in oestrum progestational proliferation results but in the presence of large doses of follicular hormone the corpus luteum hormone does not produce this effect. This may be quite significant as it has been shown by Smith (1927) and others that large doses of follicular hormone cause resorption or abortion of young in pregnant rats. Also this experiment and other available data indicate a definite quantitative relationship between these two hormones during pregnancy, the details of which we hope to present in another report.

Discussion. The point of chief interest in the experiments just described is that the progestational proliferation of the rabbit uterine endometrium seems to be the result of a combined effect of the follicular and corpus luteum hormones. Neither the follicular nor the corpus luteum extracts used were able to produce these changes when given alone. It seems as though the rabbit uterus must be under or recovering from the influence of the follicular hormone before the corpus luteum can act. The results reported here also lead us to conclude that the corpus luteum extract is not capable of prolonging the progestational condition over an extended period without the presence of the follicular hormone; that is, it seems the action of the follicular hormone is not only necessary for the beginning of the reaction by the corpus luteum extract but the follicular effect must be present if the corpus luteum extract is to prolong the progestational picture. The function of the follicular hormone would then appear to govern the growth and enlargement of the uterus, as this is the

condition characteristic of oestrum, while the corpus luteum hormone modifies the structures already formed. The follicular hormone is a growth promoting substance as indicated by the numerous mitotic figures during its maximum influence while the corpus luteum hormone, at least when given alone, does not seem to have this ability.

The idea that the follicular hormone cannot by itself produce progestational proliferation is upheld by the observations of Loeb and Kountz (1928) for the guinea pig and Corner and Allen (1929) for the rabbit. These workers were not able to produce a progestational endometrium in these animals with follicular hormone alone. That this reaction is the result of both the follicular and corpus luteum hormones is also given support by the work on the relaxation of the pelvic ligaments of the guinea pig (Hisaw, 1929) and the production of placentomata in rats and guinea pigs (Weichert, 1929). In both of these cases the animals had to be first put in the proper physiological state by the injection of follicular hormone before the corpus luteum extract could produce its effect.

Corner and Allen (1929) through the use of an ether, alcohol soluble extract of sow corpora lutea were able to produce progestational proliferation in the uterus of certain immature rabbits while others did not respond. It seems quite probable, in the light of the work reported in this paper, that those animals which gave positive results might have had sufficient follicular development to make the corpus luteum reaction possible and the negative responses were due to a lack of follicular hormone. These latter animals then were comparable to castrates.

Not only does it seem that the rabbit uterus must be under or recovering from the effects of the follicular hormone before the corpus luteum hormone can elicit the progestational reaction but it appears quite probable that an optimal quantitative relationship between the two substances may exist. This opinion is based on results for simultaneous injections of both hormones. Although rabbit 7 received sufficient corpus luteum extract to promote endometrial changes the response seemed to have been inhibited by the large amount of follicular hormone. It is possible then, in this reaction, to prevent the action of the corpus luteum by giving large doses of the follicular hormone while the follicular substance in small quantities does not prevent and perhaps promotes the changes. The corpus luteum extracts used in these experiments were prepared after the method described by Hisaw, Fevold and Meyer (in press). By this procedure an extract can be made which contains very little or no follicular hormone. This, we feel, is important as it permits a differentiation between follicular and corpus luteum reactions. A small amount of follicular hormone does not seem, however, to alter and indeed seems to aid such corpus luteum tests as the progestational changes of the rabbit uterus and the relaxation of the pelvic ligaments of the guinea pig but for such tests

as the inhibition of oestrum and the promotion of vacuolar changes in the vaginal mucosa it is decidedly advantageous to have corpus luteum extracts free of follicular hormone. Our preparation differs from that used by Allen and Corner (1929) in that it is a fat free, ether, acetone insoluble fraction. Yet there is little if any reason to doubt but what the active substance in these two extracts is the same, at least insofar as the progestational reaction is concerned. The hormone responsible for these changes seems to be soluble in ether and acetone in the presence of fats but when fats are eliminated it cannot be extracted from an aqueous solution by these solvents.

Another difference between these two extracts is the one used in this work was also able to relax the pelvic ligaments of the guinea pig while it seems quite probable that the preparation employed by Allen and Corner could not do this. This opinion is based on our repeated failures to extract the relaxative substance by methods similar to that described by Allen and Corner. It should be stated, however, that there are grounds for believing that the substance responsible for the relaxation reaction may take little or no part in promoting progestational changes in the uterus. It is possible to remove the relaxative substance from our preparation in crystalline form without impairing its ability to produce the progestational changes. This, as well as other facts (Hisaw, Fevold and Meyer, in press), suggests the probability of there being more than one hormone secreted by the sow corpus luteum.

SUMMARY

From the data presented in this paper it seems quite clear that the progestational proliferation characteristic of the rabbit uterus during pseudopregnancy is the result of a combined effect of the follicular and corpus luteum hormones. The function of the follicular hormone seems to be that of putting the uterus into a proper physiological condition so it can respond to the corpus luteum hormone. Neither of these substances can produce progestational proliferation in the castrate uterus when given alone. If, however, the castrate uterus is first brought into the condition typical of oestrum through the injection of follicular hormone and is followed immediately by corpus luteum treatment progestational proliferation results. It also seems that a quantitative relationship between the follicular and corpus luteum hormones must exist for the prolongation of the progestational picture over an extended period.

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FRICTIONAL AND KINETIC FACTORS IN THE WORK OF SPRINT RUNNING

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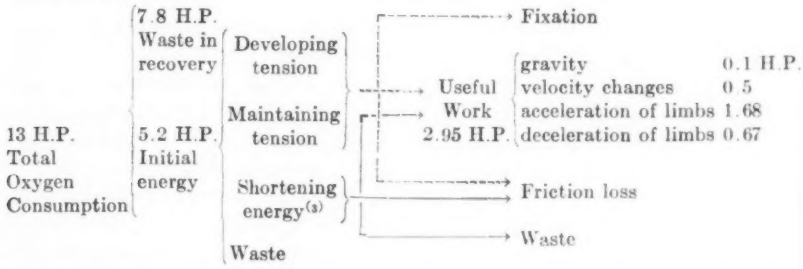
If one divides the energy equivalent of the total excess oxygen consumed as a result of a short sprint at top speed by the number of seconds occupied in the run, one finds[1] (after correcting for the start and the pull up) (cf. Sargent, 1926) that the rate of energy expenditure while running at a maximum speed is about 13 horse power for an average man. From measurements on isolated muscles (Hill, 1926) as well as from calculations made by Furusawa, Hill and Parkinson (1927) on sprinters, it may be concluded that about 40 per cent of this energy was expended during the sprint in the "initial" or anaerobic phase of muscle contraction, the remainder representing the inefficiency of recovery. The present paper is concerned with the disposition of this initial energy which is being expended at a rate of about 5.2 horse power.[2]

The conclusions reached and the problems involved are illustrated in the following diagram:



[1] Unpublished measurements from this laboratory for which I am indebted particularly to Dr. E. Fischer, Mr. H. Brody and Mr. C. I. Wright.

[2] Furusawa, Hill and Parkinson (1927) have found one man in which this figure was 8.5 horse power and Gertz by a somewhat similar method has estimated 6 to 8 horse power for the expenditure of mechanical energy while running at top speed. These men, however, were fast runners. The average speed of our runners was only 8.2 yards per second. Taking an average weight of 150 lbs. and an average propelling force of 0.75 the body weight the horse power would be $8.2 \times 3 \times 150 \times 0.80 \div 550 = 5.4$ horse power.

Some of the arrows in this diagram should be accompanied by question marks. Thus the source of the work is doubtful, whether from shortening energy or previously developed potential (tension) energy. It is also possible that the "shortening energy"[3] is partly wasted or partly used to overcome friction. The point of chief interest in this diagram, however, is the magnitude of the rate of useful work which has actually been measured from moving pictures and was found to be 2.95 horse power. The various items which are included in this figure are shown in the last column and include work against gravity, changes in velocity due to wind resistance and to bringing the foot into contact with the ground, and the kinetic energy changes of the limbs. Marey and Demeney in 1885 made an estimate of the magnitude of these various components of the work. Their estimates for the first two items agree fairly well with ours but their estimate of the kinetic energy of the limbs was 7 or 8 times too small because they measured only the average velocity of the limbs during a swing, neglected movements around the knee and elbow joints and neglected the back swings (cf. Amar, 1923, p. 504). In walking, the work against gravity is a much more important factor than in running and indeed Benedict and Murschhauser (1915) found it to be 23 per cent of the total oxygen consumption in excess of the standing value. Hill, 1927, who has discussed in many valuable papers the problems connected with rapid movements of muscles has regarded frictional loss as the limiting factor in a fast run and as in fact the only item of considerable importance. From the above diagram, however, it is obvious that, after deducting the work, only 5.2 minus 2.95 or 2.25 horse power is left to be divided between the three items of fixation energy, waste heat and frictional loss. The isometric contractions involved in making the body rigid and in fixating the joints, etc., are by no means negligible although there is no method of measuring them exactly; hence the energy left for frictional loss is not so large as has been supposed. In order to show how significant this conclusion is for the theory of muscular contraction, some further discussion of muscle viscosity is necessary.

A new method of demonstrating muscle "viscosity." The problem of "muscle viscosity" can be clearly presented as applied to man, by the following simple experiment in which the muscle tension is measured as it decreases with increasing speed of movement. The subject seats himself on a table with one leg hanging over the edge. Arrangements are made for recording variations in the angle of the knee with time as the leg swings from the knee. This is most easily done by fastening the leg to a light wheel, the horizontal axis of which coincides with the axis of the knee joint. As the wheel revolves with the leg it winds up a thread carrying a short

[3] The shortening energy is the excess heat developed when a muscle is allowed to shorten (Fenn, 1923). Thanks to Professor Hill's sense of humor this is better known in the literature as the "Fenn effect."

pointer; the thread is held taut by an elastic band. The curve traced by the pointer on a revolving drum as the leg swings indicates angles plotted against time. The slope of this curve represents the angular velocity in radians per second. If the angular velocity is plotted as a function of time the slope of the resulting graph represents angular acceleration and must be proportional at any moment to the torque applied to the leg at that time. The proportionality factor is the moment of inertia of the leg around the knee axis. This may be estimated with fair accuracy from the measurements of Braune and Fischer (1892) on cadavers and enables us to calculate the net external force exerted by the muscles at any moment in the swing. If the subject endeavors to extend his lower leg when the knee angle is 90° while the torque is measured with a spring balance it is found that he can develop a force of 19 kgm. at a distance of 43 cm. from the knee axis. If the lever arm of the extensor muscle is 4 cm. this indicates a force of $\frac{43}{4} \times 19$ or 204 kgm. If while the muscles are exerting this torque the experimentor suddenly releases the foot, the torque imparts an acceleration to the lower leg which can be measured from the graphic record. If the moment of inertia of the leg below the knee be taken as 3×10^6 gram and centimeter units, the torque producing the acceleration can be calculated (torque = moment of inertia $\times$ angular acceleration). Analysis of a quick release record obtained in this way shows that before the foot has travelled 2 cm. the force exerted by the extensor muscles (on a 4 cm. arm) has fallen from 204 to 108 kgm. and when the foot has moved 4 cm. the force has fallen to 55 kgm. When the foot has moved 6 cm. the velocity has become constant; there is no further acceleration and hence no further external force except that necessary to overcome friction in the joint.

In a similar manner a record of a free kick with the lower leg was taken. The angular acceleration was determined from the record and thence the force exerted at different times was calculated. In this case a maximum external force of about 142 kgm. was developed after the foot had moved about 1 cm. but by the time the foot had moved 5.3 cm. of the arc the velocity had become so great that a tension of only 37 kgm. could be maintained.

Critique of measurements of muscle "viscosity." These results show in a fairly quantitative way how extremely quickly the tension falls off in a muscle after it is suddenly released and how small a tension can be maintained when a muscle is rapidly shortening. This failure to develop tension while shortening may, in this case, be due partly to a reflex cessation of stimulation or a reflex inhibition and there would seem to be every reason for expecting that such a reflex would occur. On the other hand, the loss of tension during shortening may be due to some characteristic of

the muscle itself since it has likewise been demonstrated in isolated muscles (Hill, 1926). With this idea in mind it has been called "muscle viscosity." The term implies that some internal rearrangements inside the muscles are necessary before external tension can be displayed and that it is a *mechanical* resistance to these movements which prevents a muscle from re-developing tension, during shortening, rapidly enough to manifest tension externally. But in this case, the delay in the development of tension might equally well be in some chemical reaction involving the mobilization of the necessary energy for the contraction. In such a case the term "viscosity" would be inappropriate. The fact that the process of developing tension during shortening (Fenn, 1923) necessitates an extra liberation of energy (shortening energy) and that within certain limits the faster the shortening the less the extra energy developed, suggests this latter interpretation. Hartree and Hill (1928) in their most recent communication on this subject have confirmed in the main the finding that shortening under tension involves extra energy liberation and have taken it more seriously into consideration in their theories than heretofore, suggesting that it has an equal share with viscosity in determining the amount of work a muscle may do at different speeds of shortening. For the reasons here suggested it seems advisable to warn against the simple interpretation of the term "viscosity." There seems to be as yet no certain way of determining how quantitatively important "viscosity" or friction may be in muscular movements in man. By "viscosity," I mean a mechanical as opposed to a chemical delay in the external manifestation of tension.

In 1922 Hill published an important paper on the work of human arm muscles, contracting against different equivalent masses. With a small equivalent mass the contraction was rapid and little work was done and vice versa. It was found empirically that the diminution in work with increasing speeds was proportional to the speed of shortening and the interpretation was suggested that this energy which failed to appear as work was developed as potential energy but degraded into heat in overcoming frictional resistance in the muscles. While there is doubtless some truth in this interpretation, the fact that isolated muscles liberate less heat per second (i.e., for a given duration of stimulus) when shortening rapidly than when shortening slowly, makes it probable that in rapid contractions of the arm muscles less energy per second is actually developed. Hence we are at a loss to know how much of the diminution of tension with high speeds is due to diminished rate of energy expenditure and how much is due actually to frictional loss.

More recently in his experiments at Cornell, Hill (1927) (and Furusawa, Hill and Parkinson, 1927) has made use of a similar idea in studying the equation of motion of a sprint runner (cf. also Gertz, 1929). The runner

gradually accelerates until he reaches a constant maximum velocity. It is shown experimentally that his velocity as a function of time can be quantitatively explained by supposing that he is being propelled by a constant force and being resisted by a force which is proportional to his speed. This resisting force is again muscle viscosity (or "something which behaves like viscosity"). There can be no question that the equation derived on these assumptions adequately fits the facts. By means of this equation it is possible to determine the magnitude of the hypothetical propelling force; this force multiplied by the distance travelled gives the work done in the run. Since no appreciable external work is done in the run it is supposed that "the whole of the mechanical energy liberated is used in overcoming the frictional resistance of the body itself, particularly the 'viscosity' of the muscles themselves" (Hill, 1927).

This method resembles the thermodynamic method; it gives us the end results without telling us the mechanism. Assuming that it tells us correctly the total amount of mechanical energy expended it does not tell us in what way it was expended. It certainly is not all expended in overcoming viscosity. In the sentence following the one quoted above, Hill (1927) mentions the kinetic energy of the arms and legs which must alternately be created and destroyed, and uses it to show why the cost of running increases so rapidly with the speed. He does not emphasize, however, that this kinetic energy must be created in *spite* of viscosity and that a high viscosity would assist in destroying it, rather than the reverse. In fact as already mentioned, over one-half of the work as measured by Hill's method is actually used in creation and destruction of kinetic energy in the arms and legs, in changes in velocity of the whole body and in work against gravity.

There is another assumption underlying Hill's equation expressing the motion of a runner; the propelling force is assumed to be constant. The only justification for this is that the runner is making a maximal effort throughout the run. At the start however, the limbs are moving slowly and it seems likely that the force exerted (including the internal force used in overcoming viscosity) might be greater than at the end of the run when the limbs are moving at maximum speed.[4]

In case the possible inconstancy of the propelling force does not invalidate Hill's calculations we are left with the difficulty of explaining how it is possible that the items described as "useful work" should "behave like viscosity" in being proportional to the velocity of the runner. This must

[4] Isolated muscles when stimulated with a constant stimulus give off less heat when shortening rapidly under low tension than when shortening slowly under higher tension. (Hartree and Hill, 1928.) This suggests that in rapid running in man less energy is liberated per second and hence less tension exerted. The experiments are not however exactly comparable.

be the case or Hill's equation would not fit the facts as it apparently does. One would expect that the kinetic energy of the arms and legs for example would vary as the square of the velocity. An increase of velocity is attained however by increasing the length of the stride, as well as by moving the arms and legs more rapidly. Hence it is possible that there is a considerble range over which this factor may increase so nearly in proportion to the velocity as not to invalidate Hill's equation. Hill's facts therefore lend themselves admirably to the interpretation which he has put upon them but it does not seem certain that they do not lend themselves to some other interpretation equally well.

The remainder of this paper is devoted to the measurement of the work of acceleration and deceleration of the limbs. The measurement of the work against gravity and the velocity changes will be described in a later paper.

THE KINETIC ENERGY OF THE LIMBS. *Method of measurement.* The method used was the same as that originally used by Marey and Demeney, i.e., the moving picture. Due to the connection of one of us (C.A.M.) with the Eastman Kodak Company and particularly with the work of making the Eastman Teaching Medical Films, this work was much facilitated. In order to be able to make accurate absolute measurements from the film it was necessary to have the runners run behind a white wooden lattice work making a coördinate system with squares 1 meter on a side. Figure 1 will show the general arrangement used for this purpose. A telephoto lens was used in taking the photographs so that the camera could be placed 30 meters from the runners. If the runners ran ½ a meter behind the lattice work, the error in measuring their horizontal velocity from their positions in relation to the lattice was 1.7 per cent. This correction has been neglected in the calculations. The sharpness of the image on the film depends upon the brevity of the exposure. In the first film taken the exposure was about 0.003 second, but in the second film this was reduced to about 0.001 second with a corresponding improvement in definition.

Timing: Since the camera is turned by hand during the exposure the speed of the film is not known nor is it entirely regular. In order to time the pictures, wooden balls (croquet balls), 4 inches in diameter, were dropped in front of a vertical scale so that they appeared at the side of the film. The scale was graduated in tenths of a second so that the speed of the film could be readily determined. One of these balls is seen falling in figure 1.

Subjects: Two films were taken, one in May, 1928 and one in October, 1928. In the first case 19 men made each one run in front of the camera. In the second case 15 men ran each three times. The men ran one behind another as close together as convenient. Except in one or two cases they were all instructed to run at top speed, without any special sprints

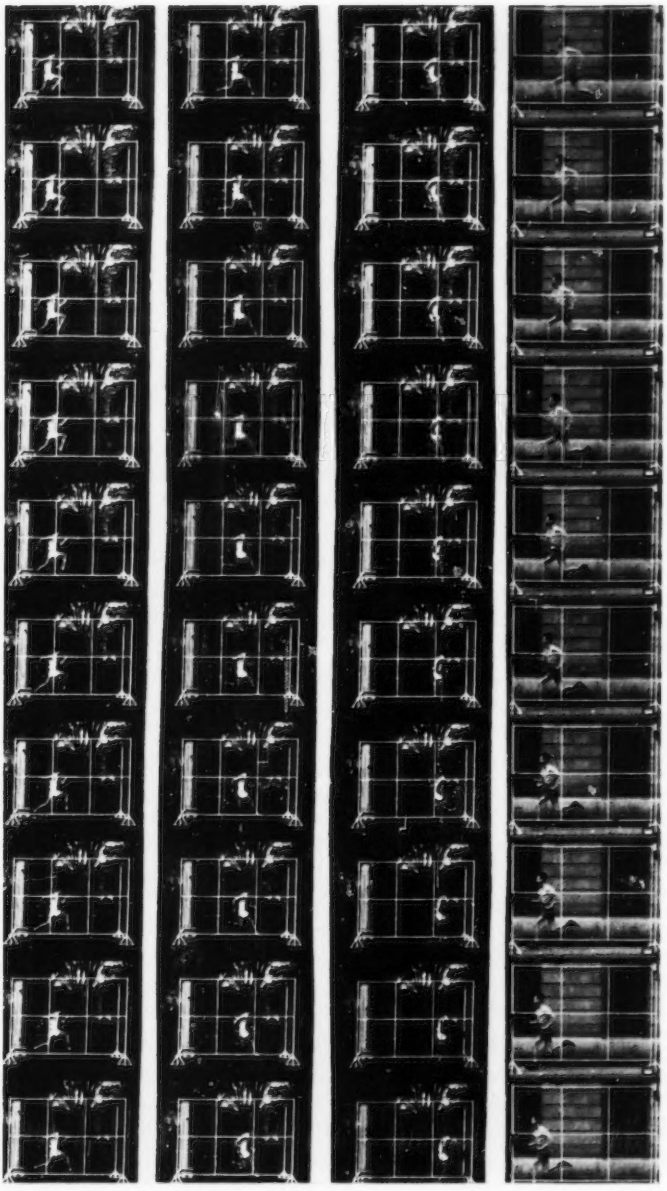


Fig. 1. Reproductions from strips of the film showing a runner behind the lattice from which measurements were made.

as they passed in front of the camera. In the first case they ran on a concrete walk and in the second case on the level turf. The general arrangement of the two films is shown in figure 1, the first 3 rows being taken consecutively from the second film and the last row from the first film. In both cases the men were members of some of the regular classes in physical education at the University of Rochester. We are much indebted to Doctor Fauver and his colleagues at the gymnasium for their coöperation in making these runners available for us. With the exception of one first rate sprinter (pictured in fig. 1) in the second group, none of the men was selected in any way. They may be regarded as a fairly representative group of college undergraduates. In order to provide fixed points for measuring the successive positions reached by a runner the men in the second group were provided with markers. The man in figure 1 shows the nature of these. One was a white cloth tied around the neck with a black spot on it. The other was a circular white tag, carrying a black spot which was supported on a stiff wire frame tied firmly around the waist in such a way that no movement in relation to the body was possible.

Measurement of the films: For purposes of projection and measurement an improvised lantern was used. In the preliminary measurements it turned out that the chief source of error lay in the buckling of the film. To avoid this the film was passed between two glass plates which could be clamped tightly together when the desired frame was brought in front of the lens. The film used was the standard $1\frac{1}{4}$ inch cinematograph film. When projected, one meter on the original lattice behind which the men ran measured 16.6 cm. For the purposes of the data to be described in this paper, measurements were made of the angles of the upper and lower arms and of the upper and lower legs. It proved to be unnecessary to measure every picture in most cases but every other picture was measured. For other purposes the vertical and horizontal positions of the markers were also determined. Where markers were absent the tip of the nose was chosen as the most definite point. It was found that the angles could be estimated within 2 degrees almost without exception. A protractor was merely laid along the longitudinal axis of the image of the limb in question and the angle read off from the intersection of a plumb line hanging from the center of the protractor.

Calculation of the kinetic energy: For purposes of calculation it is considered that the runner is standing still, as on a tread mill, and is waving his arms and legs as illustrated on the film. This method has the advantage that the kinetic energy turns out to be high in that limb where the work is being done. If the kinetic energy is calculated in relation to the ground, then the limb going backwards has very small kinetic energy although the actual effort on the part of the runner is as great in pushing it backwards as in pushing it forwards. Both methods of course lead to the

same result as far as the kinetic energy of the whole runner at any moment is concerned. But the distribution of energy over the body depends upon the point of reference chosen. The use of the runner himself as a reference point has another advantage which will be discussed later; it minimizes the calculated transmission of energy from one moving part to another. It also eliminates the necessity of knowing the momentary forward velocity of the common center of gravity of the body with great accuracy.

The kinetic energy of any part of the body with relation to the body depends upon its translational velocity with relation to the body. To this must be added its energy of rotation. Let the suffix 0 refer to the body as a whole and the suffixes 1, 2, and 3 refer respectively to the trunk, the upper leg (or arm) and the lower leg (or arm). Let m represent the weight and ω the angular velocity, v the linear velocity and s the distance to the center of gravity of the part in question and k its radius of gyration around its center of gravity. s is measured from the hip joint in the case of the upper leg and from the knee in the case of the lower leg and similarly, *mutatis mutandis* for the arm. Then the kinetic energy of the body as a whole at any moment will be (cf. Fischer and Steinhausen, 1925):

$$\frac{m_0 v_0^2}{2} + \frac{m_1 v_1^2}{2} + \frac{m_1 \omega_1^2 k_1^2}{2} + \frac{m_2 v_2^2}{2} + \frac{m_2 \omega_2^2 k_2^2}{2} + \frac{m_3 v_3^2}{2} + \frac{m_3 \omega_3 k_3^2}{2} + \text{etc.} \quad (1)$$

Here v_0 in the first term represents the velocity of the center of gravity in relation to the ground and is neglected for our present purpose. The variations in v_0 will be discussed in a later paper. v_1, v_2 and v_3 represent velocities in relation to the common center of gravity of the whole body. The values of m are calculated from the weight of the runner according to the factors determined by Braune and Fischer (1894) on cadavers. Thus if the weight of the whole runner is 1.00 the weights of the limbs, m, are as follows:

	m	s
Upper arm	0.0336	0.47
Lower arm plus hand	0.0312	0.66
Upper leg	0.1158	0.44
Lower leg plus foot	0.0705	0.61

This table also gives values of s in fractions of the length of the limb. The lengths of the limbs were measured from the photographs. The second group of runners was asked to pose for this purpose with the arms and legs conveniently bent and in a plane perpendicular to the camera for greatest accuracy of measurement. The limbs were measured from joint to joint. The lower arm was measured from the elbow to the wrist and the lower leg from the knee to the lower extremity of the tibia. The radius of gyration, k, according to the measurements of Braune and Fischer (1892) on cada-

vers may be taken with fair accuracy as 0.3 of the length of the limb. For this purpose we have to take account of the length of the hand and of the foot. Consequently we have added 13 cm. to the length of the lower arm, as measured above, this being the distance from the wrist to the first interphalangeal joint and 6 cm. to the length of the lower leg, this being the "height" of the foot as given by Braune and Fischer (1892). If therefore the length of the lower arm from the elbow to the wrist is 26 cm. the center of gravity of the lower arm plus hand is located 0.66×26 or 17 cm. below the elbow and the radius of gyration around this center of gravity is $(26 + 13) \times 0.3$ or 11.7 cm. It was not found possible to measure the lengths of the limbs with great accuracy from the photograph.

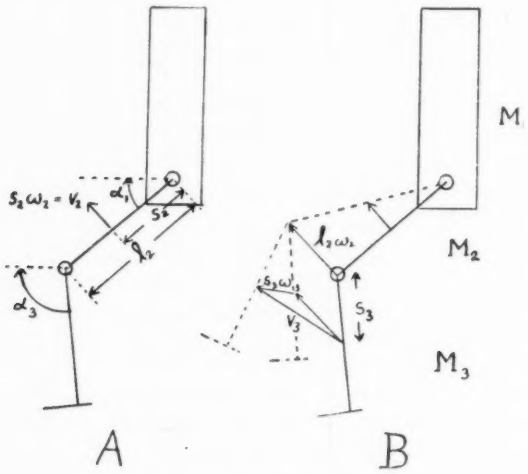


Fig. 2. Diagram of body and leg

Our most careful measurements failed to distinguish any constant difference between the lengths of the upper and lower limbs either in the arms or the legs. Thus the length of the lower arm from the elbow to the wrist is taken as equal to the length of the upper arm from the shoulder to the elbow. Likewise the length of the lower leg from the knee to the ankle is taken as equal to the length of the upper leg from the hip to the knee. All of these measurements are approximations but the errors so made cannot appreciably affect the order of magnitude of the results obtained.

In order to calculate the kinetic energy from equation (1) it is, therefore, necessary to measure from the film only the value of the angle α. From this the values of v are calculated in the following manner. Figure 2 A represents m_1 and m_2, the latter having an angular velocity of ω_2 and the

angular rotation of m_1 being neglected. Its linear velocity in relation to m_1 is therefore $s_2\omega_2 = v_2$, as represented. Its energy of translation with respect to m_1 is therefore $\frac{m_2 v^2_2}{2}$ and its rotational energy, around its center of gravity is $\frac{m_2 k_2^2 \omega_2^2}{2}$. The sum of these two factors is of course equal to $\frac{I_2 \omega_2^2}{2}$ where I_2 is its moment of inertia around the hip joint.

The calculation of the kinetic energy of the lower leg is not quite so simple (fig. 2 B). One first determines the linear velocity of the knee joint, which may be called v_l where l is the length of the upper leg. $v_l = l_2\omega_2$. Now if the angular velocity of the lower leg, $\omega_3 = o$, i.e., if the angle of the lower leg with relation to the horizontal does not change, then all parts of the lower leg, including its center of gravity will move with a velocity v_l as indicated. But if at the moment at which the knee joint moves with a linear velocity v_l, the lower leg is also changing its angle with the horizontal with an angular velocity ω_3, then the true linear velocity of the center of gravity of m_3 will be the resultant of v_l and of $\omega_3 s_3$. This resultant is v_3. The actual determination of v_3 can be done most rapidly by graphical methods rather than by trigonometry. One simply chooses a point of origin on a sheet of coördinate paper and lays off the components in their proper directions and measures the resultant with a millimeter rule. It is convenient for this purpose to let 1 cm. represent a velocity of 100 cm. per second. Knowing v_3, the kinetic energy of the lower leg is calculated by the usual formula,

$$\frac{m_3 v_3^2}{2} + \frac{m_3 k_3^2 \omega_3^2}{2}$$

A word should be included about the evaluation of the angular velocities. The data actually obtained are the angular positions of the various members at every other frame (exposure) on the film, or at every 0.016 second approximately. These angles are plotted out against the frame number (time) so obtaining *displacement curves*. These curves are smoothed out graphically with care to preserve all the significant variations. Such displacement curves for the upper and lower arms and the upper and lower legs are shown in figure 4. The angle which is measured and which is plotted in figure 4 is the angle α illustrated in figure 2, i.e., it is the angle made by the limb with the horizontal line in front of the runner. This convention has the advantage that when α is increasing the leg is going backwards and vice versa. The displacement curves are drawn for one complete cycle. The length of the cycle can be determined either from the contour of the curves or from the interval elapsing between foot-contacts with the ground. The moment when the toe leaves the ground can be

determined with somewhat more accuracy than the moment when the foot touches the ground, and this point for the two feet is preferred for determining the cycle length. Care is taken in smoothing out the displacement curves to see that they begin and end at the same angle. Since a runner does not always run with perfectly regular rhythm this sometimes involves a slight violation of the observed data but this change is altogether immaterial and doubtless represents just as fair an average cycle for that runner as the actual movement observed. It is convenient to have the two ends fit in this way in subsequent parts of the analysis. From the smoothed displacement curves the slopes are read off by a straight edge laid tangent to them at every fourth frame, or every 0.03 second approximately. It was found unnecessary to carry through these laborious calculations for every frame measured. With the angular velocities so determined the remainder of the calculation was carried out as indicated.

Now strictly speaking, equation (1) calls for the velocities of the various members of the body in relation to the common center of gravity of the whole body. Then if one can measure the velocity, v_0, of this common center of gravity one can determine the total kinetic energy of the whole moving system. It is not particularly easy to determine precisely the velocity of the common center of gravity because, as will be shown later, the body rocks backwards and forwards to some extent in running and because the position of the common center of gravity inside the body changes with the changing positions of the limbs. The effect of the latter factor can be determined and will be discussed later. It should be noted, however, that the method of calculation given above determines actually the kinetic energy of the legs in relation to the hip joint and the kinetic energy of the arms in relation to the shoulder joint. Besides this, it does not take account of the relative movements of the two shoulder joints. When the arm moves forward the shoulder joint moves forward with it. We have attempted in the case of one runner to take account of this factor by measuring directly from the film the movements forward and backwards of the shoulder joint itself. In this way it is possible to estimate that an allowance for this factor, in the case of the arms, would increase the calculated kinetic energy of the arms about 30 to 50 per cent. The effect due to the movement of the hips would be much less than this. Since the kinetic energy of the arms is rather small in comparison to that of the legs anyway, the total error involved in this factor is not large and tends to make the true figure larger (perhaps 10 per cent) than the calculated, rather than the reverse.

We have also endeavored to make allowance for the movements of the center of gravity within the body due to the varying positions of the limbs and in a number of cases we have corrected our results for the movements

of the common center of gravity within the body. Allowance for this factor sometimes makes the observed kinetic energy at any particular moment larger and sometimes smaller but in any case the difference is not large, the velocities with which the center of gravity is shifting inside the body being only about $\frac{1}{10}$ of the velocities with which the limbs are moving.

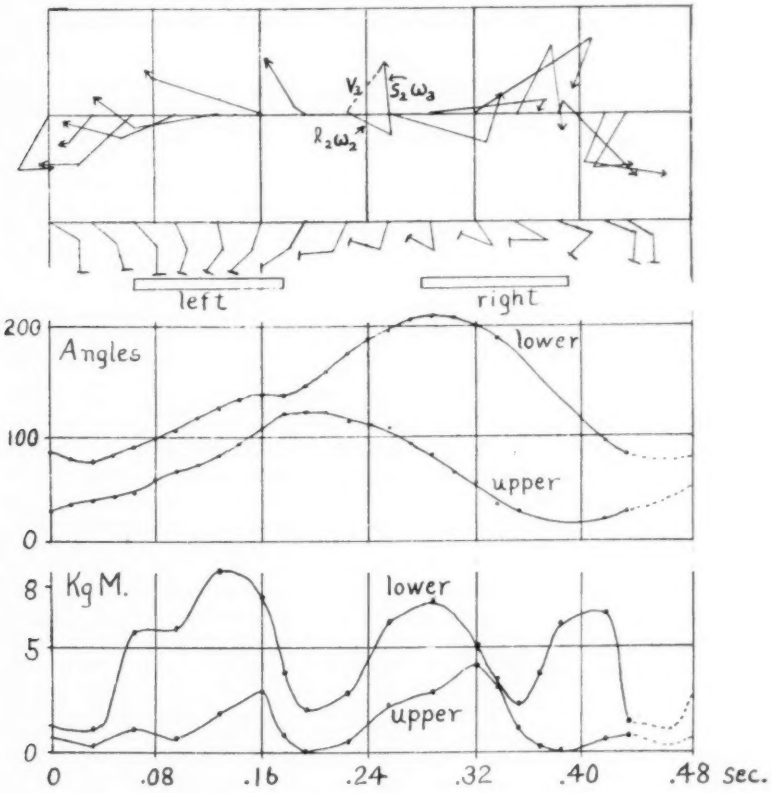


Fig. 3. Data for the left leg of runner 11. The lower graphs show the kinetic energy of the upper and lower left legs in kg. m. as function of time. The middle graphs show the angles between the leg (upper or lower) and the horizontal. The dotted portions indicate the beginning of another cycle. The arrows in the uppermost diagram show the magnitude and direction of the velocities of movement of the upper and lower leg and the resultant v_3.

RESULTS. The complete data for the left leg of one of our runners (no. 11) chosen at random, are given in table 1 and are plotted in figure 3. The first column of this table gives the number of the frame on the film.

(We have in all nearly 2000 frames each of which has been numbered on the margin in India ink for purposes of identification.) The second column gives the time in seconds as determined from the rate of fall of the croquet balls. Columns 3 and 4 give the angles with the horizontal of the upper and lower legs (femur and tibia) as measured from the film. The slopes of the displacement curve drawn through these points, as graphically determined, are given in columns 5 and 6 in degrees per frame. Columns 7

TABLE 1

Kinetic energy of left leg of runner 11

FRAME NUMBER	TIME	ANGLES		ANGULAR VELOCITY		LINEAR VELOCITY				KINETIC ENERGY					
										Upper Leg			Lower Leg		
		Upper	Lower	Upper	Lower	$v_2 = s_2\omega_2$	$l_2\omega_2$	$s_2\omega_2$	v_3	Translation	Rotation	Total	Translation	Rotation	Total
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	seconds	*degrees*	*degrees*	*degrees per frame*	*degrees per frame*	*cm./sec.*	*cm./sec.*	*cm./sec.*	*cm./sec.*	*kgm. m.*	*kgm. m.*	*kgm. m.*	*kgm. m.*	*kgm. m.*	*kgm. m.*
630	0	30	87	+3.5	−3.2	130	298	166	250	0.59	0.28	0.87	1.32	0.19	1.51
634	0.032	40	78	+2.2	+0.8	82	187	41	225	0.23	0.11	0.34	1.07	0.01	1.71
638	0.064	47	90	+4.2	+3.6	156	357	187	513	0.85	0.40	1.25	5.57	0.24	5.81
642	0.096	67	106	+3.0	+5.5	112	255	286	510	0.44	0.20	0.64	5.51	0.57	6.08
646	0.128	81	127	+5.2	+4.6	194	441	239	630	1.31	0.61	1.92	8.40	0.40	8.80
650	0.160	108	137	+6.5	+0.8	242	553	41	593	2.04	0.96	3.00	7.45	0.01	7.46
652	0.176	119	137	+3.7	+2.0	138	315	104	420	0.66	0.31	0.97	3.74	0.08	3.82
654	0.192	122	148	+0.2	+5.0	7	17	260	275	0	0	0	1.60	0.47	2.07
658	0.224	117	175	−2.7	+6.8	100	230	353	306	0.35	0.16	0.51	1.98	0.86	2.84
662	0.256	107	196	−5.6	+4.6	208	475	239	530	1.51	0.71	2.22	5.95	0.40	6.35
666	0.288	85	210	−6.5	+1.0	242	552	52	580	2.04	0.96	3.00	7.13	0.02	7.15
670	0.320	56	200	−7.7	−4.8	286	654	250	475	2.84	1.33	4.17	4.78	0.43	5.21
672	0.336	37	188	−7.0	−6.0	260	595	312	355	2.35	1.10	3.45	2.68	0.67	3.35
674	0.352	27	172	−4.2	−8.0	156	357	415	240	0.85	0.40	1.25	1.22	1.19	2.41
676	0.368	20	155	−2.0	−8.5	74	170	441	345	0.19	0.09	0.28	2.52	1.35	3.87
678	0.384	18	135	−0.7	−9.5	26	59	493	465	0.02	0.01	0.03	4.58	1.68	6.26
682	0.416	22	98	+3.0	−7.5	112	255	390	520	0.44	0.20	0.64	5.73	1.05	6.78
684	0.432	30	87	+3.5	−3.2	130	298	166	250	0.59	0.29	0.88	1.32	0.19	1.51

and 8 are calculated from column 5 by changing to radians per second and multiplying by s_2 and l_2 respectively. Column 9 is calculated similarly from column 6 for the lower leg. Column 10 is the resultant (v_3) of the velocities of columns 8 and 9 which are laid off in directions perpendicular to the angles recorded in columns 3 and 4. Columns 11 and 14 are calculated directly from columns 7 and 10 respectively and represent the translational kinetic energy of the upper and lower legs. Columns 12 and

15 are calculated from columns 7 and 9 and represent the rotational energy of the upper and lower legs respectively. Column 13 is the sum of columns 11 and 12 while column 16 is the sum of columns 14 and 15.

The values of the kinetic energy of the upper and lower legs respectively are given in columns 13 and 16 as they vary with time. These values are plotted in figure 3 in the lower two graphs. The corresponding positions of the leg are shown in the same figure. The graph begins as the left leg starts its backwards movement. The kinetic energy of the *upper leg* is seen to increase slightly until the moment when the foot makes contact with the ground. (At this moment of contact the foot is moving backwards in relation to the body but forwards slightly in relation to the ground.) Contact with the ground causes a slight check to its backwards movement and the kinetic energy falls off slightly. There is a corresponding irregularity at this point in the graph showing the angle between the upper leg and the horizontal. These angles can be measured with an accuracy of 2 degrees so that slight deviations are significant. The kinetic energy then increases again to a still higher level which is reached near the end of the backward stroke, at which point the kinetic energy falls again to zero. During the succeeding forward stroke the kinetic energy passes again through a maximum.

The kinetic energy of the *lower leg* never reaches zero but it starts at a low level as the leg starts backwards. At the point where the foot makes contact with the ground there is a slight hump in the curve, the maximum being reached toward the end of the period of foot contact with the ground. When the thigh starts forward the kinetic energy of the lower leg passes through a minimum but does not become zero because of the flexion of the knee. As this flexion continues, the knee being simultaneously carried forward, the kinetic energy of the lower leg passes through a second maximum which declines as knee flexion gives way to knee extension. As the thigh reaches its extreme forward position the lower leg tends to be thrown rapidly forward and downward thus producing a third maximum in the kinetic energy curve.

As already explained the velocity of movement of the center of gravity of the lower leg depends upon the velocity with which the knee carries it (without change in its angle with the horizontal) and the velocity with which its angle with the horizontal is changing. These two vector quantities are represented in the upper part of figure 3. Each point is represented by a jointed arrow in two parts. The starting point of each arrow represents the time to which it applies. The first joint represents the direction and velocity of movement of the knee. The second joint, terminating in the arrow head, represents the direction and velocity with which the center of gravity of the lower leg is moving because of its change of angle *with the horizontal*. The resultant of these two vectors is v_3 as

indicated on the diagram. It will be noticed that each of these vectors is drawn at right angles to the actual position occupied by the limb in question as illustrated below in figure 3. Also it will be noticed that where v_3 is large the kinetic energy of the lower leg is also large and vice versa. The rotational energy is sufficiently small to be negligible for purposes of this comparison.

Similar data from runner 1 are plotted in figure 4. Here the positions of the arms and the kinetic energy of the upper and lower arms are also represented. The displacement curves for both arms and legs showing the angles occupied in successive moments of time are also plotted. On these graphs the points represent the actual measurements taken from the screen and the curves drawn show the extent to which it is necessary to smooth out these curves before calculating slopes. In the case of this runner every frame was measured although as usual, the kinetic energy was calculated only at every fourth frame. It will be seen that the actual measurements in this case do not quite cover the period of a complete cycle so that a slight extrapolation is necessary. In this runner the kinetic energy of the lower leg reached a high peak and then fell off just as the foot left the ground. This great increase in the kinetic energy is coincident with the vigorous push backward given as the foot leaves the ground. The angular velocity of the upper leg becomes high at this moment also as shown by the sudden rise in its displacement curve at frame 25, figure 4. The low minimum in the kinetic energy reached immediately afterwards is coincident with the cessation of movement of the thigh as it turns forward and the low angular velocity of the lower leg as the ankle extends. The diminution in angular velocity of the lower leg due to extension of the ankle is seen clearly by the flat place on the displacement curve of the lower leg at about frame 25, figure 4.

It is the large distance of the lower leg from the body which makes the work necessary to swing it the most important single item in the total kinetic energy of the limbs. This work can be very appreciably diminished if the knee is flexed as it is when the leg is being brought forward for another step, for in this way the moment of inertia of the leg as a whole is much decreased. Thus, to choose a case at random, it was found that the leg of runner 2 had a moment of inertia of 18.9×10^6 gm. cm.^{2} with the leg extended (knee angle 142 degrees) while the moment of inertia was only 5.5×10^6 gm. cm.^{2} when the knee was flexed (angle 32 degrees). For this purpose the moment of inertia was calculated from the formula of Braune and Fischer (1892).

$$I = m_2 k_2^2 + m_3 k_3^2 + m_2 s_2^2 + m_3 (l_2^2 + s_3^2 - 2 l_2 s_3 \cos \beta)$$

where β is the angle between the upper and lower legs at the knee, l is the length of the part and s is measured from above downwards. It should be

noted that this formula cannot be used for calculations of the kinetic energy of the whole leg at any particular moment (using the formula

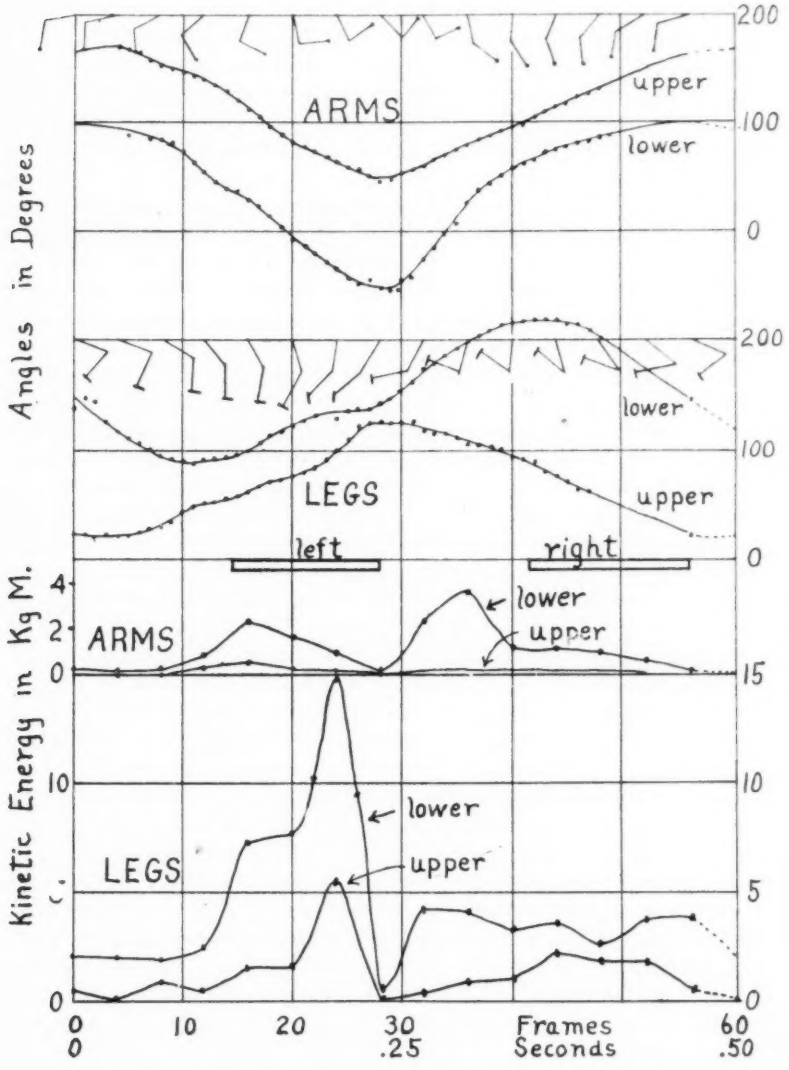


Fig. 4. Kinetic energy and displacement curves of runner 1

$I\omega^2/2$) because it does not enable one to take account of the movement of the lower leg or arm relative to the upper leg or arm; it assumes the whole limb to be a rigid body. Marey and Demeney (1885) took no account of this difficulty nor apparently of the fact that the angular velocity is not uniform throughout the stroke.

On account of the special importance of the lower leg it is necessary to consider how characteristic the lower leg curves of figures 3 and 4 may be. For this purpose similar data from 10 other runners have been plotted in figure 5, at the top of which the approximate positions of the leg are shown diagrammatically.

All of these graphs of figure 5 represent the kinetic energy changes of the lower leg. They are all arranged so that the moment when the foot leaves the ground comes at frame 30. The moment when the foot comes in contact with the ground varies slightly in different runners. In nearly every case there is an irregularity in the curve at this point. Measurements show also that there is always slight bending of the knee to break the shock as the weight of the body comes on the foot. All of these curves are sufficiently characteristic so that it is possible to tell fairly accurately in each case when the foot is on the ground from the shapes of the curve. Each of these curves shows in general three succeeding peaks which differ somewhat among themselves. (The first of these is the most characteristic and coincides approximately with the period of foot-contact.) The second peak comes as a rule at or a little before the time when the angle made by the lower leg with the horizontal begins to decrease instead of increase (marked by the first arrow on each graph) and also near the point where the forward angular velocity of the thigh is at a maximum. The third of these peaks comes approximately at the point where the thigh reaches its maximum forward position (marked by the second arrow on the graph). The last three of these graphs all come from one runner, no. 21, who was the fastest sprinter in the group, his velocity being 8.3 to 8.5 meters per second.

As a starting point in analyzing these curves and calculating the horse power expended by the runners it may be assumed that each time the kinetic energy of a limb increases there is a corresponding expenditure of energy by the muscles, and each time it decreases, a corresponding amount of energy is dissipated as heat. The total kinetic energy developed during one cycle is therefore the sum of all the *increases* in kinetic energy as taken from the curves. The sum of all these increases for all the limbs divided by the length of the cycle will give the horse power. Data so obtained from all our runners are collected in table 2. The first column gives the runner's number (no. 3 and nos. 22–35 were not analyzed). Column 2 gives the weight of the runner in kilos. Columns 3 to 6 give the increases in kinetic energy observed in the upper and lower arms during their forward

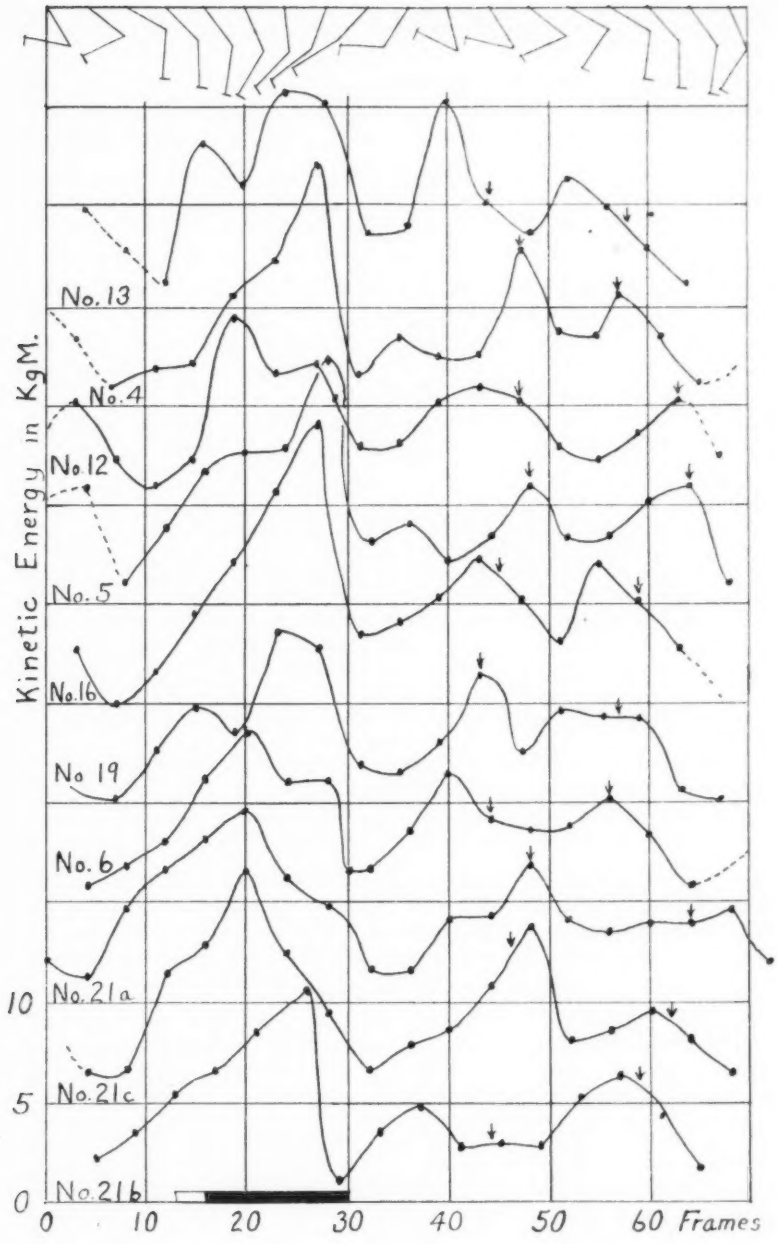


Fig. 5. The variations of kinetic energy of the lower legs of 10 different runners in 10 different cases. The moment when the foot leaves the ground is made to coincide in each case with frame 30.

and backward movements, respectively. The sum of columns 3 to 6 is given in column 7. Columns 8 to 12 give the corresponding increases in kinetic energy of the legs, upper and lower. Figures given in parentheses

TABLE 2

Increases of kinetic energy (in kilogram meters) of arm and leg during one running cycle

RUNNER	WEIGHT	ARM					LEG							ARM AND LEG	H. P.
		Upper		Lower			Upper		Lower			Sum			
		For.	Back	For.	Back	Sum	For.	Back	Back	Flex. of knee	For.	By parts	As a whole		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
	kgm.														
1	68	0.51	0.16	2.33	3.54	6.54	2.26	5.52	12.95	3.85	1.25	25.83	25.65	32.37	1.83
2	65	0.45	0.26	2.06	1.69	4.46	2.13	2.40	4.90	0.82	5.13	15.38	13.59	19.84	1.12
4	67	0.38	0.29	1.41	3.92	6.00	4.31	4.55	11.16	6.10	2.26	28.38	26.65	34.38	1.98
5	63	0.57	0.64	1.45	3.95	6.61	2.93	5.38	11.69	3.99	2.73	26.72	25.15	33.33	1.83
6	66	0.31	0.20	1.01	1.57	3.09	2.24	2.24	9.11	4.60	3.18	21.37	17.64	24.46	1.34
7	64	0.80	0.40	4.02	2.81	8.03	3.59	3.63 (2.43)	8.78	5.16	3.51 (4.03)	24.67	23.86	32.70	1.79
8	64	0.39	0.16	3.04	1.79	5.38	2.11	1.59 (6.86)	10.10	8.91	2.21 (2.45)	31.38	26.38	36.76	2.03
9	61	0.14	0.20	0.49	0.60	1.43	4.14	1.37 (1.68)	6.09	1.30	3.91 (0.88)	20.12	19.43	21.55	1.11
10	67	1.00	0.31	0.86	2.53	4.70	4.54	2.23 (1.25)	8.49	2.95	3.38	24.15	23.64	28.85	1.61
11	52	0.20	0.21	1.45	1.24	3.10	4.17	2.36	7.29	5.08	4.37	24.52	21.89	27.62	1.68
12	58	0.33	0.31	2.57	3.03	6.24	3.70	3.27	9.08	3.06	3.12 (2.79)	22.23	19.34	28.47	1.56
13	70	0.27	0.27	1.59	1.15	3.28	3.57	4.86	9.77	5.30	3.02	29.31	26.29	32.59	2.06
14	70	0.22	0.32	1.39	1.23	3.16	2.46	2.90	5.36	4.15	2.48	17.35	14.41	20.51	1.12
15	64	0.54	0.37	3.80	1.06	5.77	2.31	3.44	10.75	3.83	2.52	22.85	22.58	28.62	.62
16	73	0.12	0.18	0.97	2.87	4.14	4.11	4.65	14.30	4.36	4.22 (3.35)	31.64	30.10	35.78	1.96
17	70	1.03	0.76	3.60	4.68	10.07	5.73	7.76	17.55	3.00	5.03 (3.59)	42.42	32.90	52.49	2.75
18	70	0.40	0.24	2.77	2.72	6.13	5.26	2.90	13.86	4.30	2.69 (1.35)	32.60	31.55	38.73	1.65
19	59	0.17	0.39	0.80	4.51	5.87	2.79	3.44 (0.78)	8.53	5.29	2.07 (1.08)	23.47	23.02	29.34	1.61
20	61	0.62	0.51	2.68	3.24	7.05	2.88	3.01	9.99	2.81	1.14	21.19	21.08	28.24	1.57
21-a	64	0.35	0.18	2.37	2.83	5.73	3.22	1.65	8.66	5.15	1.09	19.77	17.17	25.50	1.49
21-b	64	0.28	0.21	3.00	1.77	5.26	3.41	2.66	9.20	3.94	3.53	22.74	17.77	28.00	1.53
21-c	64	0.45	0.17	4.08	2.47	7.17	2.44	2.02	10.35	7.34	1.63	23.78	22.13	30.95	1.81
Average.		0.43	0.31	2.17	2.51	5.42	3.38	3.35	9.91	4.33	2.93	25.09	22.82	30.50	1.68

above the line indicate extra increases associated with the making of foot contact with the ground which it was felt ought to be included. Such additional increases are shown in figure 5 for runners 13 and 19 but these have not been included in the calculations. The sum of columns 8 to 12 is given in column 13. Column 14 will be discussed later. Column 15 is the sum of columns 7 and 13 and represents the total kinetic energy developed in one leg and one arm during one complete cycle. Column 16 represents the horse power for both arms and both legs together, calculated by multiplying figures of column 15 by 2, dividing by the length of the cycle and changing units. The *average rate of energy expenditure in the arms and legs alone is seen to be 1.68 horse power.* If the total rate of energy expenditure in the sprint is taken as 13 horse power (from oxygen consumption measurements) then this represents 12.9 per cent.

Discussion. It has been assumed in the interpretation of table 2 that when the kinetic energy decreases in a limb it is not stored as potential energy but is degraded to heat. If it were stored temporarily as potential energy it could of course reappear as kinetic energy either in some other part of the body or at some other phase of the cycle in the same member. It is at this point in fact that the interpretation becomes particularly difficult. Consider first the storage of energy in some potential form.

The effect of gravity: Energy is of course stored as potential energy whenever a limb is raised. To what extent then does gravity assist in the alternating movements of running and to what extent does it modify the conclusions drawn from table 2?

The original theory of running was that of Weber (1836) who regarded the movements of the legs as simple pendulum movements. It has been conclusively shown however by Braune and Fischer for a man walking that gravity does not explain the movements and that active muscular contractions must occur to produce the accelerations observed (cf. Amar. p. 504). What is true of walking must be even more true of running where the velocities of the limbs are so much greater. Consider for example the time necessary for the leg to fall by gravity from a horizontal position in front of the body to a vertical position under the body. Let the masses of m_2 and m_3 the upper and lower legs be respectively 7000 and 4900 grams, $l_2 = 44$, $s_2 = 19$, $k_2 = 13.2$ and $k_3 = 15.0$. To simplify the conditions let the whole mass of the lower leg be considered as located at the knee joint instead of hanging vertically from it as in normal running. The moment of inertia of the whole leg is then 14.3×10^6. The torque due to gravity is $(7000 \times 19 \times 980) + (4900 \times 44 \times 980) = 342 \times 10^6$. The time to fall from the horizontal to the vertical,[5] for a compound pendulum is

[5] I am indebted to Mr. T. Tomboulian of the Department of Physics, University of Rochester, for this formula.

$$t = \frac{\pi}{2}\sqrt{\frac{I}{mgl}}\left[1 + \frac{k^2}{4} + \frac{9\,k^4}{64} + \frac{225\,k^6}{2304}, \text{ etc.}\right] \qquad (2)$$

where $k = \sin\frac{90 - \alpha}{2}$ and α is defined as in figure 2. For the case where $\alpha = 20°$ this reduces to $1.73\sqrt{\frac{I}{mgl}}$ whence the time for fall is calculated as 0.354 second. The actual time for the corresponding change in position during running is less than half this amount indicating clearly that the muscles must have contributed to the fall.

The effect of gravity needs further consideration. Its relation to the leg movements is of chief importance. In the case of the upper leg, if its mass is 7000 and s is 19 the energy available when it falls from a horizontal to a vertical position is $7000 \times 19 \times 980 = 13 \times 10^7$ ergs = 13 joules. The actual kinetic energy found in the leg after this fall in 7 different runners was 75, 33, 30, 33, 45, 42, and 46 joules or an average of 43 joules, or over times as much as could be derived from gravity. This 13 joules (1.33 kgm. m.) must therefore be deducted from the kinetic energy of the upper leg during its back stroke.

It should be emphasized that the weight of the lower leg does not serve to pull the upper leg down. Actually the upper leg would fall more rapidly if the lower leg were absent. Likewise the arm will fall to the side from the horizontal position less rapidly if a heavy weight is carried in the hand than if the hand is empty (provided friction, etc., is negligible). Its natural period as a pendulum is thereby increased. The leg is therefore pushed downwards to some extent to make contact with the ground. On the forward stroke of the upper leg the maximum kinetic energy is reached at an angle of 75° with the horizontal. At this point a negligible fraction (4 per cent) of these 13 joules has been restored to the leg in potential form and the remainder may be assumed to come from the kinetic energy it now possesses. Hence the kinetic energy as listed in table 2 requires no correction for gravity at this point.

From table 2 it appears that the upper leg develops a kinetic energy of 3.35 kgm. m. on its backward stroke and a similar amount again on its forward stroke. Of the latter, all is produced from muscular activity while of the former, it appears that 1.3 kgm. m. may be assigned to gravity.

Consider now the effect of gravity on the three peaks of the kinetic energy curve of the lower leg as illustrated in figure 5. When the kinetic energy begins to increase for the first of these peaks the foot is already practically on the ground and remains so throughout this part of the curve. Hence none of its kinetic energy as recorded in column 10, table 2 can have come from the force of gravity. When the toe leaves the ground the lower leg is raised and at the same time its kinetic energy rises to a maximum.

This maximum is reached at about the same time that the lower leg reaches its maximum elevation. Hence the work done against gravity in raising the leg should be added to the kinetic energy produced in this knee flexion (column 11, table 2), which has an average value of 4.33 kgm. m. Measurements show that in the case of runner 1 the center of gravity of the lower leg is elevated 33 cm. during this interval and the work done is therefore 33 cm. $\times$ 980 $\times$ 4790 gms. or 15.5 $\times$ 10^7 ergs or 1.58 kgm. m. The total energy expended by the muscles in moving the lower leg at this period of the cycle is therefore 4.33 + 1.58 or 5.91 kgm. m. The next peak in the kinetic energy of the lower leg comes when the thigh reaches its maximum forward position. During the interval between this and the preceding peak, the center of gravity of the lower leg remains practically on a level because, as the knee rises, the ankle falls a corresponding amount. Hence none of the potential energy of position which the lower leg possessed at the point of maximum knee flexion can have been transformed into kinetic energy to contribute to this second peak. The figures in column 12, table 2, require therefore no correction.

On the whole, therefore, corrections for gravity entail a deduction of 1.3 kgm. m. for the upper leg and an addition of 1.58 kgm. m. for the lower leg. Both of these are small amounts and the difference is negligible in comparison to other errors. The effect of gravity on the arms may be neglected as a relatively insignificant item in the total balance sheet for the body.

Storage of energy in tendons and muscles: From the above considerations it is obvious that the storage of energy as potential energy of position and its reappearance as kinetic energy is not an important factor in evaluating the mechanical horse power of sprinting. Is it possible, however, that there could be such storage of energy in stretched tendons and muscles? It seems that the tendons can be dismissed because the actual positions reached by the limbs in swinging are not sufficiently extreme to stretch the tendons without the participation of actively contracted muscles. Likewise the resting muscles could not exert appreciable tensions in the positions occupied by the limbs at the end of their strokes. If the limb were stopped entirely by frictional forces all its energy would be degraded to heat and there would be none to store. Suppose therefore that muscles must contract and exert a tension F against a moving limb for a time t such that Ft equals the decrease in momentum of the limb. In doing so the muscle is stretched and might be supposed to have stored up a certain amount of potential energy. It cannot retain this store of energy, however, without continuous contraction. If it has any potential energy it is continuously losing it at a certain rate and continuously redeveloping it. The balance between those two determines the amount of tension maintained and the energy of maintenance. A muscle may therefore be said to

"charge storage" at a rate which would expend the energy value of the stored energy many times over in a few seconds. In the movements of running, a study of the movies shows that the *tension is maintained during the reversal of direction of motion of the limbs* for the acceleration is practically constant during this period. Presumably in this case then the maintenance expenditure is less than the cost of redeveloping the tension and the back stroke must necessarily be somewhat quicker if the tension is already developed.

But however that may be, the energy which the muscles save by thus avoiding the necessity of redeveloping a certain tension for the back stroke is no measure of the amount of potential energy corresponding to that tension; which is the question at issue for the present discussion. Nor can potential energy be measured by the work which the muscle will do when it is allowed to shorten (cf. Fenn, 1923).

The area of the length tension diagram has often been regarded as so much potential energy but one can never recover anything like this amount of energy as work (not over 30 per cent), nor is it certain that the work which is recovered actually came from previously developed potential energy and not from other chemical breakdowns taking place during the performance of the work. The excess production of energy observed when work is done favors the latter view. The absence of such excess heat in single twitches has been cited, however, as proof that in this case at least the work must have come from previously developed potential energy (Hartree and Hill, 1928). Although I do not yet feel convinced that this absence of excess heat in single twitches is universally true (since I have observed variations in the initial heat production of muscles with change of load even when stimulated by single twitches under Ringer's solution) nevertheless the result, if true, does not preclude any other hypothesis. One observes simply more energy production during the contraction phase when work is performed, as compared to an isometric contraction, and correspondingly less during relaxation. It may be said that the performance of work accelerates part of the chemical breakdown which otherwise does not appear as heat until relaxation. We have no real means of knowing *where* this same relaxation heat was during the isometric contraction phase—perhaps still in the form of chemical potential energy. Moreover the division of the heat into contraction and relaxation phases is not altogether precise and in any case the relaxation heat is not over half the length-tension area corresponding to the isometric tension developed (Hartree and Hill, 1928). In short many facts fit beautifully into the theory that an isometrically contracting muscle possesses mechanical potential energy like a stretched spring and does work by this means. But it is equally true that the known facts do not altogether preclude another hypothesis according to which the energy needed for muscular work is developed during the actual period of shortening.

In conclusion it appears that during the reversal of a limb, the muscles are continuously innervated so that tension is maintained, redevelopment of tension for the back stroke is avoided and the energy equivalent of a certain amount of oxygen is saved. Such a saving of oxygen does not mean however a saving of mechanical energy. The kinetic energy observed in the return stroke may nevertheless have to be redeveloped *de novo* in spite of the fact that the necessary tension is still there. As a guess it might be said that the storage of energy could not be over 25 per cent of the kinetic energy of the limb before reversal.

Work of deceleration. The work of deceleration is work necessary to stop a moving limb; it is tension exerted while a muscle is being stretched, or negative work. Measurements have been made in man of the extra oxygen consumption involved in such positive and negative work. Chauveau (1901) found for example that negative work involved 52 per cent as much energy as positive work. Zuntz gave a figure of 40 to 45 per cent. Cathcart (1922) has reported a considerably higher figure of 71 per cent even after allowing for the energy used in performing movements without a load. Positive work is done in spite of viscosity and negative work with the aid of viscosity. If the external work is A and the viscosity is x, and if the oxygen consumption is assumed proportional to the work, then, using Cathcart's figure, $A + x/A - x = 100/71$ and $x = 1/6$ of A. If Chauveau's figure (52 per cent) is used, then viscosity work is $5/16$ of A. Similarly Zuntz's figure (40 per cent) gives $3/7$ of A. This estimate is of somewhat doubtful value partly because we never know exactly what the antagonistic muscles are doing in movements in man and partly because we have no proper assurance that the measurements were made either during a steady state or so as to cover completely the period of recovery. Moreover, these figures do not take into account the fact that a muscle loses tension at less than the isometric rate while being stretched and at more than the isometric rate while shortening, so that the necessary rate of tension redevelopment or the heat production is less during stretching than during shortening (Fenn, 1923). Correction for this difference would make the work of viscosity less than $3/7$ of A, so that this is probably an upper estimate at the speed employed in the experiments of Zuntz. Also the work against viscosity will vary much with the speed of movement and this factor has not been controlled and is at a maximum in the rapid movements of sprinting. For this last reason it seems safest to choose the lowest of these estimates, (i.e., 40 per cent) in allowing for the work of deceleration of the limbs. *Hence if the rate of work in accelerating the limb is 1.68 horse power, the work of deceleration would be 0.4 × 1.68 or 0.67 horse power,* which would seem to be a conservative estimate.

Transfer of energy across body: When the foot is in contact with the ground in running it is occupied in exerting a force F backwards on the

ground for time, t, so that Ft represents the momentum, mv imparted thereby to the body. Much of this goes for example not directly into the body but into the other leg which is being carried forward at a greater velocity than the body. When this leg reaches the end of its forward stroke its momentum must be shared with the body as a whole according to the law of the conservation of momentum. In this way momentum can be transmitted about the body from one part to another, by a series of *inelastic impacts*. It is pertinent to inquire therefore to what extent kinetic energy can disappear from one limb only to reappear in another and so be counted twice in estimating the horse power of sprinting. A partial answer to this question may be suggested in the following manner.

If I_1 is the momentum of inertia of the body around a transverse axis through the two hip joints and I_2 the moment of inertia of the leg around the same axis then, as the leg swings forward with an angular momentum of $I_2\omega_2$, it is checked by the hamstring muscles and an angular momentum $I_1\omega_3$ is imparted to the body, such that

$$I_2\,\omega_2 = (I_1 + I_2)\,\omega_3 \tag{3}$$

both limb and body moving then together with an angular velocity ω_3. The energy gained by the body is then $\frac{I_1\omega_3^2}{2}$ and that lost by the leg is $\frac{I_2\,(\omega_2^2 - \omega_3^2)}{2}$ and the fraction, f, of the energy lost by the leg, which is transferred, is

$$f = \frac{I_1\,\omega_3^2}{I_2\,(\omega_2^2 - \omega_3^2)} \tag{4}$$

From a small cadaver studied by Braune and Fischer (1872, table 2) the following data are obtained.

	WEIGHT	T	e	I
Body and head	23790	10.57×10^6	30.14 cm.	32.2×10^6
Leg	7840	4.87	32.74	13.2×10^6

I was calculated from the formula $I = T + Me^2$ where M is the weight, T the moment of inertia around the center of gravity and e the distance of the center of gravity from the hip joint. Using these values of I_1 and I_2, ω_3 may be calculated in terms of ω_2 from equations (3). Thus $\omega_3 = \frac{13.2}{45.4}\,\omega_2$ and $\omega_3^2 = 0.085\,\omega_2^2$. This value for ω_3^2 can now be substituted in equation

(4) and the fraction of energy transferred becomes equal to $\frac{32.2}{13.2} \times \frac{0.085}{0.915} = \frac{1}{4.4}$. This would seem to be a maximum figure for several reasons. It assumes the knee completely extended and rigid. When the knee is bent I_2 may be 5×10^6 whence the fraction transferred is only $\frac{1}{8.5}$. Moreover when one leg is going forward the other leg is going back so that the body is being twisted simultaneously in opposite directions. Thus neither leg will be able to twist the body and hence no energy can be transferred. The body is thus steadied by the opposite limbs in such a way that it behaves as if its moment of inertia or mass were much larger than it is. Hence the energy transfer is far less.

In this connection it is worth pointing out that the body itself has been chosen as a reference. If we had chosen the ground instead of the body as a reference point the problem of this transfer of energy would have involved an inelastic impact between two bodies moving at different speeds, i.e., the body of mass m_1 would be moving at velocity v_1 in relation to the ground while the leg of mass m_2 would be moving, during its forward stroke, for example, with a velocity v_2. For the sake of simplicity its angular movement may be neglected. Then $m_2 v_2 + m_1 v_1 = (m_1 + m_2) v_3$ and using values for m_1 and m_2 given above and taking v_1 and v_2 as 7 and 9 meters/sec. respectively v_3 becomes 7.5 and the fraction of energy transfer is $\frac{m_1}{m_2}\left(\frac{v_3^2 - v_1^2}{v_2^2 - v_3^2}\right) = 0.87$. As the leg swings forward it has a relatively high velocity and hence a high kinetic energy in relation to the ground. The above figure shows that at most (7/8) of this energy is not degraded to heat but is transferred to the body which is thus accelerated in stopping the leg. This change in velocity of the body is real and has been measured and its magnitude will be discussed in a later paper. Obviously when the ground is used as a point of reference the danger of counting energy twice is considerable.

The present paper deals, however, only with the kinetic energy of the limbs. To disappear from one limb and reappear in another, energy must be transferred from leg to body and again from body to leg. Utilizing similar methods in this case, it may be found that $\frac{1}{2.4}$ of energy from a body moving with angular velocity v_3 may be transferred to a stationary leg. Thus the total energy transferred from leg to leg is only $\frac{1}{4.4 \times 2.4}$ or about $\frac{1}{10}$.

There remains the important question of transfer of energy from the upper to the the lower leg or vice versa. In particular take the case where the thigh is moving forward and is checked while the lower leg continues to move forward. It may be thought of as "snapping" forward like a whip. Whether in this case there is any appreciable energy transfer needs no discussion for it can be answered experimentally from the data at hand. When the kinetic energy disappears from the upper leg a corresponding amount should appear in the lower leg. A study of figure 4 shows that in this runner, at least, the successive increases of kinetic energy in the lower leg cannot be derived in appreciable degree from the upper leg. Instead the kinetic energy contents of both upper and lower legs tend to increase and decrease more or less together. To test this point for all runners the kinetic energy of the whole leg was determined for each point in the running cycle by adding together the figures obtained for upper and lower legs separately. The successive increases in kinetic energy of this combined curve were then determined and added together. The resulting sum is shown in column 14 of table 2. These figures are all slightly less than the corresponding figures of column 13 which were obtained by adding together the separate increases of the upper and lower legs. On the average, however, the difference is small, 22.8 kgm. m. as compared to 25.1 kgm. m. or a 9 per cent difference. Hence *at most 9 per cent of the kinetic energy could have been counted twice.* This does not necessarily mean that 9 per cent *was* in fact transferred from upper to lower leg or vice versa. Possibly therefore the figure 1.68 horse power for the arms and legs is 9 per cent too high and the true figure is 1.53 H.P. This small reduction, however, is completely offset by the previous estimate that the movements of the shoulders if allowed for would increase the observed kinetic energy of the limbs as a whole about 10 per cent.

These considerations make it appear probable that the figure obtained for the kinetic energy changes of the limbs is a fair representation of the actual conditions. Sideways movements of the body have necessarily been neglected as well as the contortions of the face and the contractions of the body muscles, etc. Altogether it seems that the actual output of mechanical energy by the body in sprinting is as large in relation to the oxygen consumption as when the work is measured, for example, on a bicycle ergometer. It would seem as if these mechanical factors had been unduly neglected in preference to viscosity in considering the work of running.

SUMMARY

1. The problem of muscle viscosity is discussed in its relation to the physiology of sprinting in order to show that the available evidence does not preclude the possibility that the actual external work of sprinting (exclusive of work done against viscosity) is a large fraction of the total energy expended.

2. This conclusion is then verified by measurements of moving pictures of sprinters whereby the kinetic energy of the limbs could be calculated and plotted as a function of time.

3. An average sprinter is incurring an oxygen debt at the rate of 13 horse power while he is turning out mechanical work at the rate of 2.95 horse power or with an efficiency of 22.7 per cent. This includes work against gravity (0.1 horse power), changes in velocity (0.5 horse power), acceleration of the limbs (1.68 horse power), and deceleration of the limbs (0.67 horse power). It excludes contractions of facial and body muscles, sideways movements of the body and work against viscosity or internal friction.

4. The discussion is concerned chiefly with the possibility of storage of mechanical energy in the muscles and tendons and the transfer of momentum and energy from one part of the body to another. It is concluded that these complications do not seriously interfere with the accuracy of the figures obtained for the acceleration and deceleration of the limbs.

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PHYSIOLOGY OF THE CORPUS LUTEUM

VI. The Production of Progestational Proliferation of the Endometrium of the Immature Rabbit by Progestin (an Extract of the Corpus Luteum) after Preliminary Treatment with Oestrin

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In previous publications[1] we have described the preparation and effects of corpus luteum extracts upon castrated rabbits and guinea pigs. These extracts produce in the adult, recently castrated female rabbit progestational proliferation of the endometrium, a reaction which occurs normally in the rabbit's uterus during early pregnancy and known to be de[illegible] associated with the growth of the corpus luteum. When the doe [illegible] mated from 15 to 20 hours before castration these extracts maintain normal growth and implantation of the embryos and development of the fetuses even to term. Thus castrated pregnant rabbits are enabled to continue the pregnancy normally when under the influence of this hormone, and because of this property of aiding gestation we have suggested for this hormone the name *progestin*. These same extracts likewise produce in castrated female guinea pigs the special sensitization of the endometrium necessary for the production of experimental deciduomata, a reaction for many years known to be inseparably linked with the young corpus luteum.

In the second paper of this series it was shown that extracts which produced with perfect regularity a complete proliferation of the endometrium of the adult castrated rabbit would only occasionally produce such a response in the immature female; in most cases doses several times the minimum for the adult animal produced little or no proliferation in the immature animal. This difference in reaction between the immature and

[1] Previous papers of this series are listed in the bibliography as follows: I, Corner, 1928; II, Corner and W. M. Allen, 1929; III, Allen, W. M. and Corner, 1929; IV, Tatelbaum and Goldstein, 1929; and V, W. M. Allen, 1930. For the extensive literature of ovarian and placental hormones, see Allen, E. and Doisy, 1927.

the adult animal was very puzzling and led us to discontinue the use of the young animal in the study of this hormone. The ovaries seemed not to be responsible since no difference could be detected between the ovaries of those animals which reacted fully and those which did not respond. Therefore we were led to assume that, inasmuch as the corpus luteum never occurs in the normal animal until after sexual maturity, pure corpus luteum preparations would have no effect on the immature animal, and that the variability in response was due to the varying amounts of oestrin which the crude extracts were known to contain, the uterus of the immature rabbit being very sensitive to oestrin.

TABLE 1

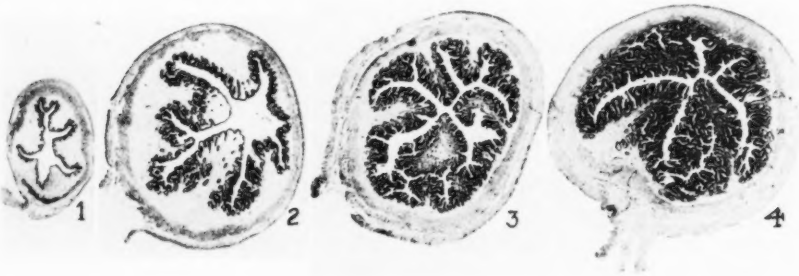
RABBIT NUMBER	AGE AT AUTOPSY	WEIGHT AT AUTOPSY	METHOD OF TREATMENT	PROLIFERATION AT AUTOPSY
	days	*grams*		
X-150	101	1362	3.5 rat units of oestrin per day for 10 days, then 0.3 cc. of progestin per day for 5 days	++++*
X-172	55	576	8 rat units of oestrin per day for 5 days, then 8 rat units of oestrin and 0.3 cc. of progestin per day for 5 days	+++
4	55	608	Same as X-172	++++
X-151	101	1475	0.3 cc. of progestin per day for 5 days	None
X-153	140	1475	Same as X-151	+
X-173	55	426	Same as X-151	+
X-175	55	652	Same as X-151	None
X-168	66	1078	Same as X-151	None

* For illustrations showing the standard degrees of proliferation as indicated by these + signs, see W. M. Allen, THIS JOURNAL, 1930.

If this explanation is correct, then immature rabbits whose uteri have first been brought into the adult state by the injection of oestrin, should react with regularity to injections of progestin in a manner exactly similar to the response of the adult animal. That such is the case is shown by the following experiments.

The first group of experiments consisted of eight immature rabbits varying in weight from 576 to 1475 grams and in age from 55 days to 140 days (table 1). Each of the five controls (X-151, X-153, X-173, X-175, X-168) was given 0.3 cc. per day of a crude preparation of progestin for five days and then autopsied on the day following the last injection. In three of the animals (X-151, X-175, X-168) there was no growth nor proliferation, and in two (X-153, X-173) (fig. 2) there was some growth and a very slight amount of proliferation. Thus three times a dose which

was known to be adequate to produce a ++++ proliferation in the adult castrated animal caused little or no proliferation. One animal (X-150) was given 3.5 rat units of oestrin per day for 10 days, and then during the next 5 days 0.3 cc. of the same extract of progestin as given the controls. At autopsy on the day following the last injection the uterus was fully proliferated. The other two does, only 55 days old and still nursing, were given 8 rat units of oestrin per day for 5 days, and then during the next 5 days 8 rat units of oestrin together with 0.3 cc. of the same progestin extract per day. On the day following the last injection the animals were



Figs. 1-4

Fig. 1. Section of uterus of X-192, showing the complete absence of growth or proliferation following the injection of 0.1 cc. of progestin per day for 5 days. Compare with figure 3. × 7.

Fig. 2. Section of uterus of X-153, showing growth and a trace of proliferation produced by 0.3 cc. of progestin per day for 5 days. × 7.

Fig. 3. Section of uterus of X-195, showing growth and a complete proliferation (++++) produced by 2 rat units of oestrin per day for 7 days, followed by 2 rat units of oestrin and 0.1 cc. of progestin per day for 5 days. Compare with figure 1. × 7.

Fig. 4. Section of uterus of X-181, showing growth and extreme proliferation (+++++) produced by 8 rat units of oestrin per day for 6 days, followed by 8 rat units of oestrin and 0.2 cc. of progestin per day for 5 days. × 7.

autopsied, and after sectioning the uteri were found to show +++ (X-172), and ++++ (X-174) proliferation. In this case three times the minimal adult dose produced proliferation in all the animals first treated with oestrin, and little or no proliferation in all animals untreated with oestrin.

The second group of animals (table 2) consisted of six immature rabbits varying in weight from 681 grams to 1193 grams, the exact age being unknown. Three of them (X-178, X-180, X-183) were given 0.2 cc. of crude progestin preparation per day for 5 days and autopsied on the day following the last injection. Microscopic sections revealed that all showed

considerable growth, one a + (X-180) and two a ++ (X-178, X-183) proliferation. The other three animals (X-179, X-181, X-182) were given 8 rat units of oestrin per day for 6 days and during the next 5 days 8 rat units of oestrin together with 0.2 cc. of a crude progestin preparation per day. On the day following the last injection the animals were autopsied and the uteri submitted to histological study. Each of the three showed

TABLE 2

RABBIT NUMBER	WEIGHT AT AUTOPSY	METHOD OF TREATMENT	PROLIFERATION AT AUTOPSY
	grams		
X-179	1193	8 rat units of oestrin per day for 6 days, then 8 rat units of oestrin and 0.2 cc. of progestin per day for 5 days	+++++
X-181	908	Same as X-179	+++++
X-182	739	Same as X-179	+++++
X-178	1193	0.2 cc. of progestin per day for 5 days	++
X-180	681	Same as X-178	+
X-183	1021	Same as X-178	++

TABLE 3

RABBIT NUMBER	W IGHT AT AUTOPSY	METHOD OF TREATMENT	PROLIFERATION AT AUTOPSY
	grams		
X-190	1419	2 rat units of oestrin per day for 7 days, then 2 rat units of oestrin and 0.1 cc. of progestin per day for 5 days	+++
X-191	1193	Same as X-190	++++
X-194	1193	Same as X-190	++++
X-195	1475	Same as X-190	++++
X-192	1362	0.1 cc. of progestin per day for 5 days	None
X-193	1419	Same as X-192	+
X-196	1247	Same as X-192	None
X-197	1647	Same as X-192	+

growth to approximately the adult size and a proliferation (+++++) (fig. 4) greater than that ever seen in the adult normally pregnant rabbit. In this group twice the minimal adult dose produced very great proliferation (+++++) in all those animals treated with oestrin and only moderate (++) proliferation in those untreated with oestrin.

The third group of animals (table 3), varying in weight from 1193 grams to 1647 grams, were given the minimal adult dose of progestin and

a much smaller amount of oestrin than the animals of the preceding groups. Four of them (X-192, X-193, X-196, X-197) were given 0.1 cc. of progestin per day for 5 days, and then autopsied on the day following the last injection. In two of these (X-192, X-196) (fig. 1) there was no growth nor proliferation, and in two there was some growth and a very slight proliferation (+). The other four animals were given 2 rat units of oestrin per day for 7 days, and then 2 rat units of oestrin and 0.1 cc. of progestin per day for 5 days. The animals were autopsied on the day following the last injection and the uteri submitted to histological study. Three of them (X-191, X-194, X-195) (fig. 3) showed ++++ and one (X-190) a +++ proliferation. In this group therefore the minimal adult dose of progestin produced little or no response in the four rabbits untreated with oestrin, whereas it produced complete proliferation in the four treated with oestrin.

SUMMARY. All of the 10 animals treated with oestrin and progestin showed complete proliferation (+++ or ++++) of the endometrium. Of the 12 animals given progestin only, 5 showed no growth nor proliferation, 5 showed some growth and traces of proliferation, and 2 showed some growth and a moderate proliferation (++).

The corpus luteum preparations (progestin) were made according to the method described by W. M. Allen (1930), the preparation being stopped at the end of stage II, e.g., after the removal of the phospholipids by acetone. The oestrin preparation used in groups I and II was made in this laboratory from follicular fluid of the sow by the method of Thayer, Jordan, and Doisy (1928) and was standardized on four rats, the minimum amount which would produce the oestrous smear in all four of the rats following three injections being considered the rat unit. One rat unit when standardized in this manner was equal to 0.048 mgm. of solids and was injected in an aqueous medium. The oestrin preparation used in group III was made by Parke, Davis & Co. from sows' follicular fluid and was standardized here in the same manner as that made in the laboratory. All standardizations of oestrin were completed after the conclusion of the experiment.

In studying the uteri of the rabbits six blocks were taken from each uterus, one from the upper portion, one from the middle segment, and one from the lower part. These were fixed in Bouin's fluid and prepared as usual for histological study. It was found necessary to take several blocks in this manner because frequently in the immature rabbits there was some variation in the degree of proliferation, especially in those animals given corpus luteum only. In these animals the uterus in general often remained infantile in size, while a small portion, sometimes only 5 mm. long, might be distinctly enlarged and show a slight proliferation. This peculiar reaction seemed to demand the taking of representative

blocks from both horns to guarantee an accurate knowledge of the exact degree of proliferation. The variability of proliferation in the animals treated with oestrin and progestin was very slight, never more than the difference between a +++ or ++++ proliferation.

From a casual glance it might seem that proliferation was due to the oestrin instead of the progestin, since the animals getting progestin only did not respond, whereas those that received progestin plus oestrin did respond. This was not the case, however, for it has been adequately shown by Corner and W. M. Allen (1929), Courrier and Masse (1928), and Courrier and Potvin (1926), that oestrin does not produce any proliferation either in the immature or adult female rabbit. To further verify this statement one animal of the first group was explored after the treatment with oestrin and before the treatment with progestin and oestrin and a sample of the uterus removed for study. This showed the usual growth following oestrin injections but no proliferation.

Hisaw (1929) has shown that his corpus luteum extracts are ineffective in the relaxation of the female guinea pig's symphysis unless the animal has first been under the influence of oestrin, either made by her own ovaries or injected. This need of oestrin to bring the uterus into a state of susceptibility to the corpus luteum seems to be in definite agreement with the series of immature rabbits described above. All of the adult rabbits which we have used as described in previous publications have been in a state of oestrus (the domestic rabbit is in that state almost continuously) and castrated only for a few hours before the first injections, so their uteri are probably already under the influence of oestrin before the progestin is given. These results together with those of Hisaw also lend experimental support to the statement of Novak (1928), deduced from theoretical considerations of reproduction in general, that extracts of the corpus luteum might be expected to have little effect in the human being unless the uterus was first prepared by the injections of follicular fluid (oestrin).

It has been stated by some that there is an antagonism between oestrin and the corpus luteum, but the above series of experiments certainly lends no support to this hypothesis; since the series is too small for a quantitative study of the relations of oestrin and progestin, it cannot be definitely stated that large doses of oestrin either would or would not inhibit the action of progestin. Courrier (1928), however, has shown that large doses of oestrin (230 rat units) have no inhibitory effect on the ability of the rabbits' own corpus luteum to produce progestational proliferation.

CONCLUSIONS

Progestational proliferation can be induced regularly in the uterus of the immature rabbit weighing from 575 to 1647 grams by the injection of

progestin if the uterus is first brought under the influence of oestrin. If, however, the animals are not treated with oestrin before the administration of progestin, only a very small percentage of them respond to progestin treatment.

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CAFFEIN AND DIURESIS IN MAN

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The object of the following experiments was 1, to determine to what extent caffein is a diuretic in a healthy human subject, and 2, to trace the diuretic effect of caffein when it is competing to influence water excretion with various amounts of sodium chloride and with pituitrin.

Adolph and Ericson were able to distinguish between two stimuli that increase the rate of water output, finding that pituitrin inhibited completely the extra excretion of water following the ingestion of pure water, but not the extra excretion of water following the ingestions of salts in solutions more concentrated than the maximal excretory concentration. Which kind of stimulus is given by caffein?

METHOD. The present experiments were performed on a healthy normal man weighing 74 kgm. They were started usually in the morning, one hour after breakfast. Urine was collected every half hour as long as the experiment lasted. One or more normal or control periods preceded the ingestion of one of the various solutions. The following urinary characteristics were measured: Volume, chloride content by the Volhard method as modified by Harvey, chloride concentration, specific gravity by means of a hydrometer. Body weight was also recorded to 0.1 kgm. at convenient intervals.

Care was taken by drinking plenty of water the night before, that the hydration of the body was complete previous to each experiment, the thirst experiment excepted.

THE RESULTS of the chief experiments are presented in plates I and II. The data are plotted in the middle of the time interval in which the sample accumulated in the bladder.

The effect of caffein on water-output under ordinary conditions, represented in figure 3, is a moderate increase in the rate. The increase is also seen to follow a second dose of caffein when given a short time after the first (fig. 11). With water in excess in the body, produced by drinking 1 liter of tapwater, the effect of caffein is indistinguishable, as is seen by comparing figures 1 and 2. The total excessive excretion of water

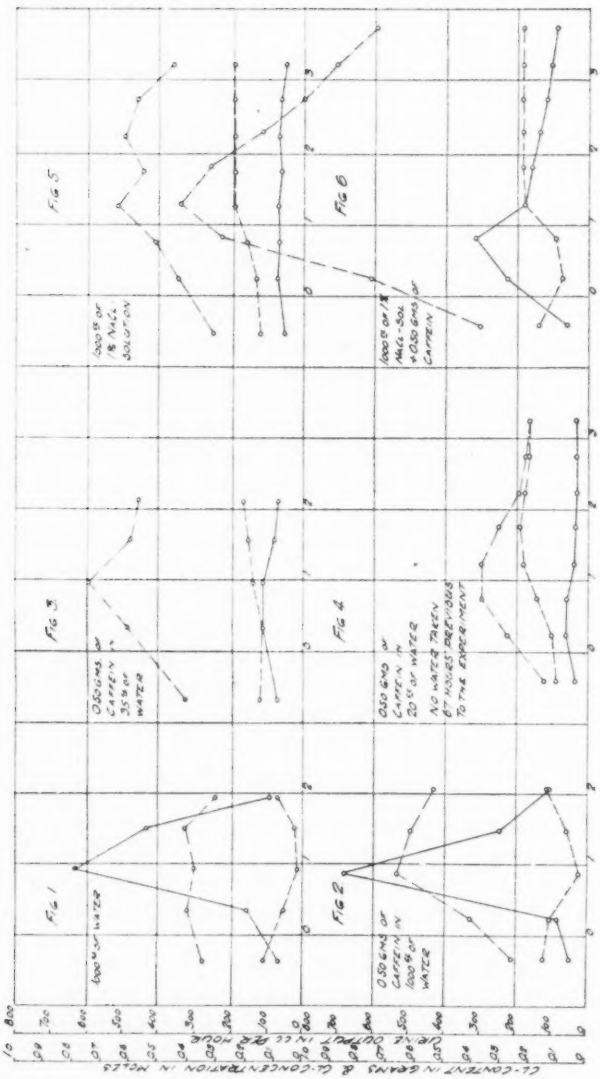

Plate I. ——— Urine output, - - - - - Cl.-content, - - - - - Cl-concentration. Abscissae: Time in hours. Ingestions of solutions at times marked 0.

amounted within two hours to about 60 per cent in both cases. Under a condition of dehydration, produced by omitting water from the diet as far as possible for 67 hours previous to the experiment, the effect of caffein, as represented in figure 4, is definite but smaller than with normal hydration (fig. 3). In this case the body was deprived of water to such an extent that the ingestion of 2,800 cc. of water in the course of three hours following the experiment caused no diuresis.

When water is taken in the form of a 1 per cent NaCl solution the effect of caffein, as represented in figures 5 and 6, is an initial diuresis of about 250 cc. above that of the control experiment. This initial diuresis is characteristic; it is the diuresis which follows the ingestion of pure water. After one hour the diuresis subsides to some extent to take a more evenly decreasing course, lasting more than three hours. Without caffein there is barely a significant increase of water excretion in spite of the large ingestion of fluid (fig. 5). This experiment was done three times in all, with similar results. One of them is represented in figure 12, the caffein in this case being taken 1 hour before the solution. In two experiments, in which solutions of 10 per cent NaCl were ingested, no such effect was observed, the only result being that the diuresis started slightly sooner when caffein was taken. (These experiments are not recorded here.)

The effect of caffein on chloride-output is shown in the same graphs. Under the usual chloride intake of the normal diet the effect of caffein is to increase the rate of Cl-output, as represented in figure 3. A similar result is noted in figure 2 when 1 liter of water was ingested with the caffein. The effect of caffein on Cl-output under a condition of thirst, as represented in figure 4, is again a considerable increase in the rate of output. The Cl-concentration is here seen to increase from the very beginning of the experiment, as is the case also in figure 5, but this is not a feature of other experiments. The Cl-concentration in figure 4 reaches a maximum within two hours after the onset of excessive Cl-excretion.

When a 1 per cent NaCl-solution, which is approximately isotonic with the blood, is ingested, the effect of caffein on Cl-output is again a marked increase as shown in figures 5 and 6. A similar result is obtained when the NaCl-solution is taken in large amounts the day previous to the ingestion of caffein as shown in figure 11. In this experiment the Cl-concentration reached a higher level than in any other. In two experiments, the same two that are referred to above, in which 10 per cent solutions of NaCl were ingested, the only effect of caffein on the Cl-output was to increase the excretion slightly sooner.

Relation of water to chloride. The experiments show that under all conditions tested, caffein causes an increased rate of output of both chloride and water. The effect is indubitable but can be classified as only moderate.

How is this effect brought about? Does caffein primarily affect one

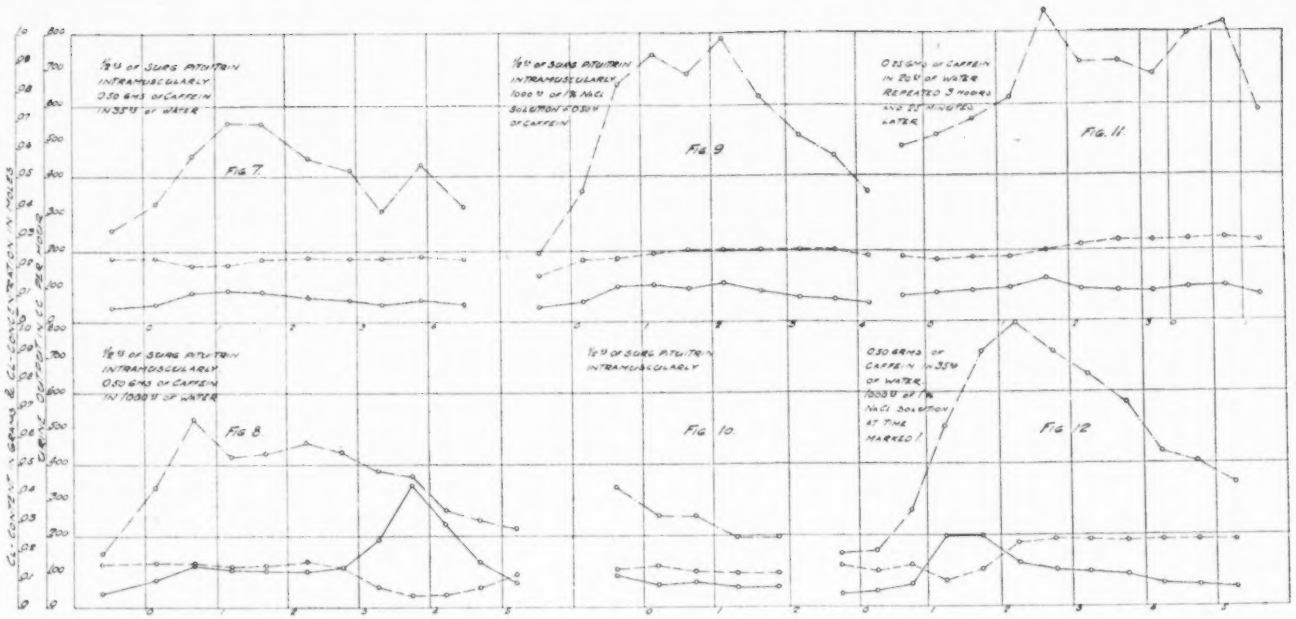

Plate II. ——— Urine output, ·-·-·-·- Cl.-content, - - - - - Cl-concentration. Abscissae: Time in hours. Ingestions of solutions at times marked 0.

factor, the other being only a consequence of this? Or does it affect the excretion of chloride and the excretion of water independently? If chloride is the primarily affected substance, it would be expected to call forth water as a carrier; but it would then be expected that the Cl-concentration would increase at the beginning of the diuresis. This is the case only in figures 4 and 5, in which the relative availability of chloride and water may have been different. If, on the other hand, water is the primarily affected substance, a washing out of chloride is a consequence to be expected, provided that chloride is readily available. If this were the case, how should the increase in Cl-concentration which is shown to persist after the peak of water output be accounted for? It seems probable that the diuretic effect of caffein is a double one, chloride and water being independently affected.

Effect of pituitrin. Is it possible to distinguish more definitely between these factors? It seemed possible that pituitrin might be used as a tool to separate them. A short series of experiments was designed in which pituitrin was injected intramuscularly simultaneously with the ingestion of caffein. Some of the results are presented in plate II.

The general effect of pituitrin on the experimental subject is shown in figure 10. Without any ingestion of water, as represented in figure 7, caffein is seen to exert its effect practically undisturbed in spite of the injected pituitrin. When 1 liter of water is taken, as in figure 8, the pituitrin delays for three hours all of the diuresis caused by the water.

When a 1 per cent NaCl-solution was taken, as in figure 9, one sees that the initial diuresis in figure 6 is absent in figure 9; in other words, the initial diuresis is inhibited by pituitrin. There remains however a definite, slight but lasting diuresis similar to that caused by caffein under ordinary conditions. This shows that two distinct diuretic influences are exerted by caffein, as was suggested by the earlier experiments.

The increased rate of Cl-output is little affected by taking pituitrin along with the caffein. The excessive Cl-output is now relatively greater than the increased water output, for the Cl-concentration attains a high level that is evidently a maximum for the conditions we are dealing with.

Discussion. We have thus two phases of the diuresis caused by caffein, one phase that is inhibited when pituitrin is injected, and one phase that is not inhibited by an injected dose of pituitrin. The first phase has to do with water alone, the second phase has to do with both water and chloride. This is shown by the fact that during the first phase the Cl-concentration may drop while the water-output increases.

As to the seat of these actions we can only say that one part of the effect, that concerned with water directly, is most likely directed toward the same elements in the body that are affected by pituitrin. Kunz and Molitor are inclined to refer at least a part of the effect of pituitrin to the tissues

in general. The second part of the effect, which has to do with chloride and in which the diuresis is not inhibited by pituitrin, is possibly a more general disturbance of the chloride and water equilibrium in the tissues. Fröhlich and Zak have shown that purine diuretics cause an increased water exchange in the tissues of cats and rabbits.

SUMMARY

Caffein has a small diuretic effect in a healthy human subject. Besides the increased rate of water excretion, there is an increase in the rate of chloride excretion. Both these increases occur also in a state of dehydration produced by taking dry food.

The diuretic effect of the caffein increases in the presence of excess water in the body only when the water is taken in form of an approximately isotonic NaCl-solution. The condition of the body is then comparable to that in some pathological edemas.

The diuretic effect has two phases, one that is completely inhibited by pituitrin, the seat of action of which has not yet been determined, another that is not affected by pituitrin, and possibly is concerned with a general disturbance of chloride and water equilibrium in the tissues.

I take pleasure in acknowledging my great indebtedness to Dr. E. F. Adolph for his inspiring advice and his kind criticism.

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STUDIES IN DECEREBRATION

V. The Tonic Activities of a Decerebrate Animal Exclusive of the Neck and Labyrinthine Reflexes

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According to Magnus (1) and his school, the static postural reflexes which are present in a decerebrate animal consist of labyrinthine and neck reflexes. Therefore, if the neck reflexes are abolished by section of the suitable cervical posterior roots (1C-2C-3C Magnus) and if the labyrinths are removed, only the muscle proprioceptive tonic reflexes remain.

When the neck and labyrinthine reflexes had been destroyed, Magnus found marked decerebrate rigidity in the three animals he described. In general it may be said that no change in tone followed a change in the position of the head in relation to the body or space (neck and labyrinthine reflexes). The "vertebra-prominens" reflex was present in all animals. Magnus differentiates it from a neck reflex, which is obtained by bending the neck forward at the occipito-atlantoid joint. When, however, the entire neck was flexed forward upon the breast, marked diminution in tone was noted in all of the extremities, in contrast to the diminution of tone only in the forelegs which follows a neck reflex produced by anteflexion of the head, at the occipito-atlantoid joint. Only a trace of a change was observed in the tone of the extremities upon turning the head in two of the animals and in them it was found that a very few fibers remain unsevered. In one animal these were found in the second cervical and in the other in the third cervical posterior roots. The head was held upright in all three animals and the extremities were outstretched so that the animals were able to support their weight upon them.

If the distribution of tone in an animal with the labyrinths removed and the cervical posterior roots sectioned is compared with a decerebrate animal deprived of the neck and labyrinthine reflexes, it is found that in the former the forelegs are always held in flexion. It is surprising that when the reflex activities which produce extension normally in the forelegs are destroyed decerebration should again produce such a position. If this is true, it follows that the body reflexes themselves possess this among other functions. Yet, it is strange that we should always find predominant

extensor tone present in such decerebrate animals since it is said that these body reflexes usually occur from asymmetrical stimulation and are followed at times by an increase in flexor tone and at other times by an increase in extensor tone, depending upon the nature of the stimulus.

In otherwise normal decerebrate animals, flexor rigidity has been noted rather frequently. Maintained flexion of the arm in decerebrate monkeys has been described by Brown (2) and by us (3). Richter and Bartemeier (4) found lasting flexor rigidity in the decerebrate sloth, whose normal activities include hanging dependent from trees. The pattern of rigidity in such animals follows the distribution of tone present normally. Unless masked by extreme rigidity resulting from other reflex activities, one should expect to find in a decerebrate animal that distribution of tone normally present in that part of the nervous system which remains intact.

In their descriptions of a decerebrate labyrinthless animal other workers always noted the presence of extreme extensor tone in the outstretched forelegs. In a former publication (5) we described the results of destruction of the labyrinths in animals decerebrated by the anemic method (6). When suspended such an animal assumed a good decerebrate rigidity in the fore and hind legs. Shortly afterward the head began to droop and the rigidity lessened in the forelegs. At this time if the head was extended passively and then suddenly released it dropped into a position of flexion and the forelegs were flexed convulsively in all joints and were abducted. At the same time the hind legs were extended backward more rigidly. This positon would be retained indefinitely until the head was extended passively or until some phasic reflex was elicited (fig. 1). From this it would appear that the labyrinths exert a strong influence upon the extensor reflex of the neck which tends to produce a fixed position of the head in extension with a consequent marked extensor rigidity in the forelegs. The play of labyrinthine tone upon the neck muscles seems likewise to be sufficient to reinforce the extensor tone of the forelegs even when the head is flexed passively in an ordinary decerebrate animal. On the other hand, the tone in the hind legs seems far less dependent upon the labyrinthine reflexes and much more upon the neck reflexes. If it is true that strong extensor tone in the forelegs is produced through the neck reflexes, either when the head is extended or when the neck reflex centers are played upon by labyrinthine reflexes, it should follow that when the labyrinths and the peripheral mechanism of the neck reflexes are destroyed the distribution of tone will depend upon the particular stimulus and the particular group of muscles of the body or area of skin so stimulated through the remaining reflex activities. The same would hold true for the hindlegs even to a greater degree because they seem to be influenced less by the labyrinthine and more by the neck reflexes.

In his effort to determine the peripheral reflex arc of the neck reflexes

Magnus was concerned with the destruction of such reflex activities as were observable in a normal or labyrinthless animal. When he was able in such animals to destroy these reflex activities, he concluded that the peripheral sensory arc was contained in the first three cervical posterior roots. It does not follow, however, that some activities, which may not be observable in such animals, may not persist in the decerebrate animal.

Because we felt that the previously described pattern of rigidity in decerebrate animals with the labyrinths removed and the first three cervical posterior roots severed did not agree with our conception of the integration of the reflex activities of a decerebrate animal, we decided to sever the first four cervical posterior roots, to destroy the labyrinths and to decerebrate a number of cats. These procedures were carried out upon seven animals. Three of these were atropinized for the purpose of another study

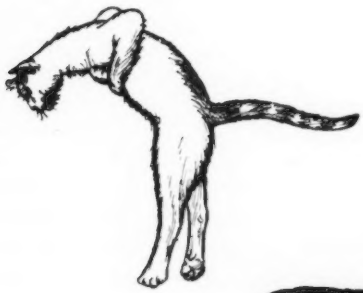

Fig. 1

Fig. 2

Fig. 1. Labyrinthless decerebrate animal.

Fig. 2. Decerebrate animal with labyrinths destroyed and the first four cervical posterior roots severed bilaterally.

and the conclusions contained herein, which would hold for all of the animals, are based upon the observations of the remaining four cats. The labyrinths were destroyed by a method previously described (5). The cervical posterior roots were cut and the animals decerebrated by the anemic method. In all instances the cervical posterior roots were severed a number of weeks prior to further operative procedures. No evidence of a spinal cord lesion was present and the roots were found to be severed completely at autopsy. The operation upon the labyrinths was performed upon two of the observed animals several days before decerebration and in the others upon the same day.

It will be necessary only to describe certain activities which were found to be different from the normal decerebrate animal. Contrary to the description of Magnus, the forelegs were in varying degrees of flexion and

the hind legs were moderately extended. The head was not held supported when the animal was suspended but there was no flexion of the forelegs when the head was allowed to fall from a passively extended position. Neither was there an increase in the extensor tone of the hind legs under this condition. Although the forelegs were always in a position of flexion and the hind legs often slightly flexed, and certainly never strongly extended, attempts to flex either fore or hind legs further produced good extensor rigidity. Often when the legs were very slowly extended and suddenly released, they fell a certain distance rapidly, then stopped and retained the new position indefinitely. Similarly, if an extremity became flexed as the result of a withdrawal reflex, it remained in this position. The resistance to slow passive movement was often of a type similar to that experienced in patients suffering from paralysis agitans. Never was it necessary to "break" the rigidity. The extensor rigidity was insufficient to hold the weight of the animal although repeated pressure against the paws developed a strong extensor rigidity. Similar to our labyrinthless decerebrate animals the reflex activities of the forelegs to nociceptive stimuli were increased. There were marked withdrawal, contralateral thrust and swipe reflexes. In the hind legs the reflexes were so active that a number of vigorous kicks would follow a single pinch of a paw. The phlyctenular, pinna and sneeze reflexes were as active as in a cerebellumless animal. The slightest stimulus to the animal would produce a marked respiratory response. Of course, no neck or labyrinthine reflexes could be obtained by turning the head. The "vertebra-prominens" reflex was absent in all of the animals (fig. 2).

The outstanding features of this preparation, therefore, are the position in flexion of the forelegs; the diminution of extensor tone in all of the legs; the relatively greater degree of extensor rigidity in the hind legs, and the marked activity of the nociceptive reflexes.

As was to be expected from our former experiments upon the labyrinths, we found that when the tonic neck and labyrinthine reflexes were destroyed, extensor tone in the forelegs was markedly diminished; that the pattern of rigidity was more or less equally distributed between flexors and extensors unless one or the other was asymmetrically stimulated. Further, that in the absence of the neck reflexes extensor rigidity in the hind legs was lessened considerably but was present to a greater degree than in the forelegs. The picture, therefore, was that of a mechanism influenced through the stimulation of the body reflexes and not masked by an overwhelming extensor tone. Although muscle proprioceptive reflexes were sufficient to produce considerable sustained and increased tone, at times, in flexion, the extreme degree of extensor rigidity observed ordinarily in a decerebrate animal was notably absent.

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THE CALCIUM IN THE SERUM IN JAUNDICE

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There is no agreement at present regarding either the quantity or the condition of the calcium in the blood in jaundice. The first students of this problem (King et al., 1909, 1911) reported that in dogs there was a moderate increase in the calcium content of the whole blood after ligation of the common bile-duct. At the same time, the urinary excretion of calcium was increased with a consequent negative calcium balance. They interpreted these results as indicating a mobilization of calcium to neutralize a toxic action of the bile pigments. Because of this supposed combination between bilirubin and calcium they considered that whereas the calcium content of the blood was increased there was nevertheless a functional deficiency in calcium. Lee and Vincent (1915) and Walters (1921) have since stressed this point in its relation to the delayed coagulation time in jaundice, and to the preparation of these patients for operation. Cantarow, Dodek and Gordon (1927) studied the calcium in the whole blood and serum of patients with jaundice. The calcium content of the serum was not decreased, but nevertheless they thought it possible that functional deficiency was present.

Snell, Greene and Rowntree, (1925) Walters and Bowler (1924) and Zimmerman (1927) did not find changes in the calcium content of the serum in experimental obstructive jaundice or in cases of jaundice. Halverson, Mohler and Bergeim (1917) and Koechig (1923) reported that the calcium in the serum is slightly reduced in jaundice although the latter stated his belief that this reduction is apparent rather than actual and the result of a loss of calcium in the preparation of a protein-free filtrate for analysis. Kirk and King (1926) and Emerson (1928) reported moderate reductions in the total calcium of the serum, but found greater reductions in the ultrafiltrable or diffusible fraction. Buchbinder and Kern (1927) did not observe changes in the calcium content of the serum after ligation of the common bile-duct in adult dogs; in puppies, however, there was a progressive decrease. Growth was arrested and in one animal clinical signs of rickets developed. These authors also noted reduction in the calcium content of the serum in three cases of jaundice.

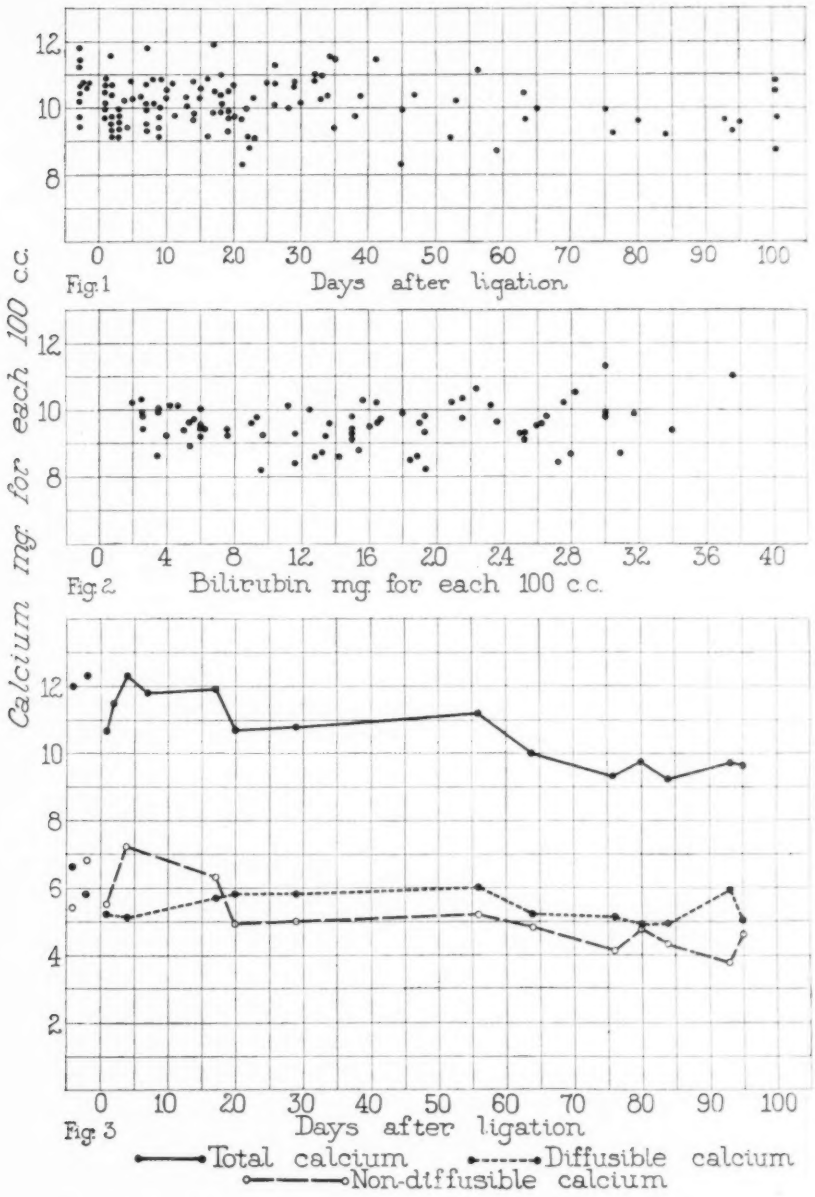

Fig. 1. The calcium content of the serum in dogs with experimental obstructive jaundice in relation to the duration of the biliary obstruction.

Fig. 2. The calcium content of the serum compared with the bilirubin content of the serum in patients with jaundice.

Fig. 3. Changes in the total, diffusible and non-diffusible calcium in the serum of a dog with experimental obstructive jaundice.

Because of the uncertainty with regard to the changes in animals, and the limited number of patients that have been studied, we have reinvestigated this problem, both experimentally in dogs and clinically in cases of jaundice. A sufficient number of observations has been made to determine, by means of statistical analysis, the normal range of variation in the calcium content of the serum and the changes produced by biliary obstruction and jaundice. The calcium content of the serum was determined in ten adult dogs by the Clark-Collip (1925) modification of the method of Kramer and Tisdall. The common bile-duct was then doubly ligated and severed aseptically under ether anesthesia. The effect of the resultant biliary obstruction was then followed by the determination of the calcium content of the serum at weekly intervals. Single determinations were made in an additional series of fifteen animals in which the common bile-duct had been tied and jaundice had been present for thirty days or more. One hundred eight determinations were made. As a control, 100 determinations were made on 100 normal adult dogs without regard to age, sex, or time of year.

TABLE 1

Calcium content of serum

	NUMBER OF INDIVIDUALS	NUMBER OF DETERMINATIONS	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION
			mgm. per cent	*mgm. per cent*	*mgm. per cent*
Dogs:					
Normal	100	100	10.77	0.05	0.78
Obstructive jaundice (final values only)	20	20	10.14	0.11	0.74
Total	25	108	10.16	0.10	0.72
Patients without jaundice:					
Men	64	87	9.82	0.06	0.71
Women	64	77	10.12	0.05	0.64
Total	128	164	9.96	0.04	0.69
Patients with jaundice:					
Men	19	32	9.45	0.10	0.65
Women	26	43	9.71	0.08	0.58
Total	45	75	9.60	0.06	0.63

Figure 1 shows that as time goes on there is very little change in the calcium content of the serum of adult dogs in consequence of biliary obstruction. In some instances a slight decrease in the calcium was noted, but in other instances there was a slight increase, and normal readings were usually obtained even after long-continued obstruction, 100 days or more. Frequency distribution curves on both series of determinations

showed a symmetric distribution according to the laws of normal probability (table 1). The mean value for the calcium content of the serum in the control series of normal animals was 10.77 ± 0.05 mgm. for each 100 cc. with standard deviation of 0.78 mgm. The mean value for the entire series of determinations on animals with obstructive jaundice was 10.16 ± 0.05, with a standard deviation of 0.72, and almost identical figures (10.14 ± 0.11 with a standard deviation of 0.74) were obtained when only the final reading for each dog was considered.

A study of seventy-five determinations of the calcium content of the serum was made in a group of forty-five cases of jaundice of various clinical types. As a control, 164 determinations on 128 hospital patients were made. This represents a miscellaneous group of cases although care was taken to exclude all cases of tetany, rachitis, nephritis, diarrhea, or diseases of the thyroid or parathyroid glands, and so forth, in which a disturbance in calcium metabolism may be present.

TABLE 2

Ages

PATIENTS	CASES	MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION
		years	*years*	*years*
Without jaundice:				
Men	64	43.6	1.05	12.4
Women	64	37.4	1.11	13.1
With jaundice:				
Men	19	52.5	1.57	10.15
Women	26	46.6	1.58	11.95

All determinations were combined for the computation of the mean and of the standard deviation of the frequency distribution of the calcium content of the serum in the normal and jaundiced subjects, regardless of the number of subjects involved. This process is justified in so far as the present series is concerned because the comparison of the distribution constants for the first or second determinations showed them to be the same. The frequency used in the computation of the probable error of the mean was that of the number of individuals involved, in order to judge the significance of the difference between the mean values obtained in normal and jaundiced subjects.

The results obtained from the study of patients were similar to those obtained experimentally from the study of dogs. There was no apparent correlation between the calcium content of the serum and the intensity of the jaundice as measured by the bilirubin content of the serum (fig. 2). As in dogs, the frequency distribution followed the curve of normal

probability in both series of determinations. The mean value for the calcium content of the serum in the control series was 9.96 ± 0.03 mgm. for each 100 cc. with a standard deviation of 0.69 mgm. In the series of patients with jaundice the mean value was 9.60 ± 0.05 with a standard deviation of 0.63.

Much has been written regarding the state of calcium in the blood and significance of changes in the so-called active, ionized, uncombined, dialysable or diffusible fraction, in explaining physiologic effects assumed to be the result of calcium deficiency. There has been no agreement as to the exact chemical characterization or the most desirable method of determining this fraction, a question we wish to consider in full at some future time. The method of Moritz (1925) as modified by Updegraff, Greenberg and Clark (1926) and by us, gives uniform and comparable values for the so-called diffusible calcium in the serum. We have, therefore, used this method as an empiric index in a study of the state of the calcium in the serum in jaundice.

Two cubic centimeters of serum were dialyzed against 5 cc. of distilled water for five to eight hours in an apparatus similar to that of Updegraff, Greenberg and Clark. A pressure of 150 mm. mercury was uniformly used. The collodion dialyzing sacs were made from a solution of Du Pont's parlodion dissolved in a mixture of alcohol, ether and ethylene glycol, as suggested by Pierce (1927). The addition of ethylene glycol greatly simplifies the preparation of a series of sacs of uniform permeability. At the end of the period of dialysis the calcium was determined in 5 cc. of the diffusate by the Clark-Collip modification of the Kramer-Tisdall method. The results were calculated on the assumption that the diffusible calcium was uniformly distributed throughout the system and no correction was made for either the change in the protein concentration in the sac or for the passage of fluid into the dialysate. There was no significant difference between results so calculated and those calculated by the method of Updegraff, Greenberg and Clark, and any error so introduced is more than compensated for by the difficulties incident to measuring with accuracy the volume of fluid on the inside and outside of the sac.

The changes shown by this method in the total, diffusible and non-diffusible calcium in the blood serum of a dog after ligation of the common bile-duct are shown in figure 3. All fractions seem to be equally affected by the development of the jaundice. Thirty-five readings on twenty-five normal dogs and thirty-nine readings on twelve dogs with jaundice were made. In both groups the diffusible calcium for the most part varied between 45 and 60 per cent, with a median value of 52 per cent in each. Similar readings were obtained in a study of the diffusible calcium in the serum in a series of twenty-five tests in a group of twenty-three patients with jaundice (table 3). The difference between the diffusible calcium

was not significant either in amount or in percentage of the total among these patients and in a similar group of normal persons.

COMMENT. A consideration of the relation of the calcium content of the serum to the prolonged coagulation time frequently observed in obstructive jaundice is beyond the scope of this paper. The effect of the intra-

TABLE 3

Diffusible calcium in cases of jaundice

CASE	TOTAL SERUM CALCIUM	DIFFUSIBLE SERUM CALCIUM	DIFFUSIBLE CALCIUM	SERUM BILIRUBIN	COAGULATION TIME		DIAGNOSIS
	mgm. for each 100 cc.	*mgm. for each 100 cc.*	*per cent*	*mgm. for each 100 cc.*	*minutes*	*seconds*	
1	9.32	5.01	54	25.0	8	30	Stone in common duct
2	9.42	4.51	48	6.3	9	20	
3	10.26	4.10	40	2.5	11	45	
4	10.02	5.03	50	3.5	7	30	
5	9.82	4.44	45	26.5	7	40	
6	10.62	4.49	42	22.4	8	30	
7	10.18	4.42	43	2.0	6	30	
8	9.22	5.17	57	4.0	6		
9	10.10	5.24	52	11.2	9	30	Stricture of common duct
10	9.62	4.37	46	9.0	9		
11	9.42	4.17	45	2.6	22		
12	9.36	4.80	51	5.0	8		
13	10.10	4.44	44	4.7	9		
14	10.16	5.53	54	20.9	25	15	Carcinoma of pancreas
15	10.16	4.59	45	16.4	26	30	
16	8.60	4.31	50	12.8	10		
17	9.62	5.32	55	16.5	13		
	9.52	5.88	62	16.0	10	20	
18	9.62	5.46	57	23.5	10	20	
19	9.42	3.93	42	6.0	8		Intrahepatic jaundice
20	9.22	3.89	42	6.0	8	20	
21	9.56	4.58	48	5.3	8	30	
22	9.90	5.54	56	30.0	8	30	
23	10.96	5.82	53	37.5	8		
	11.28	6.10	53	30.0	7		

venous administration of calcium chloride in shortening the coagulation time in some jaundiced patients is well known and the wide use of this practice by surgeons is evidence for its therapeutic value. In jaundiced patients there is no significant correlation of the amount of diffusible calcium, the serum bilirubin and the coagulation time. The administration of calcium chloride by vein to these patients may shorten the coagulation

time without affecting either the bilirubin or the total amount of calcium in the serum, and the percentage of diffusible calcium is not affected by this procedure. This is perhaps to be expected when one considers the small amount of calcium introduced and the relatively large mass of calcium already present in the blood and tissues. It is apparent that the administration of calcium salts does not have a direct effect on the coagulation of blood, but rather operates through the medium of some more complicated mechanism.

The experiments of Buchbinder and Kern have emphasized the importance of the age factor in determining the effect of biliary obstruction on calcium metabolism. They observed defective calcification of the bones and progressive reduction in the calcium content of the serum in puppies after ligation of the common bile-duct. These changes do not occur in adult dogs or at least not to the same extent, although there may still be some tendency in this direction. In some experiments, as in that shown in figure 3, there was a slight reduction in the calcium content of the serum of adult dogs following prolonged biliary obstruction. There was progressive reduction in the serum proteins from 7.1 to 5.4 per cent, during the course of the experiment. This change may be a factor in explaining the reduction in the calcium content of the serum. Peters and Eiserson (1929) in particular have emphasized the rôle of the protein concentraton in determining the calcium content of the serum. The mean calcium content of the serum was lower in the jaundiced animals, 10.16 mgm. for each 100 cc., than it was in the normal control group, 10.77. Although this reduction in the mean is sufficient to be of statistical significance it is of no apparent physiologic moment in so far as any individual animal is concerned.

Likewise there was a slight reduction in the mean calcium content of the serum of patients with jaundice (9.60 mgm.) when compared to that in a control series of hospital patients (9.96 mgm.). This was true both when each series was considered as a unit and when the two sexes were considered separately. However, the average age of the jaundiced patients was approximately nine years greater than that of the control group (table 2).

Greisheimer, Johnson and Ryan (1929), in particular, have emphasized the reduction of the calcium content of the serum with increasing age. This factor, therefore, would seem to provide as satisfactory an explanation of the slight differences between the two groups of patients as does the jaundice. Here, too, the changes in the mean values for the calcium content of the serum, although great enough to be of statistical significance, so far as the group as a whole is concerned, are certainly of no clinical significance with regard to the prognosis or treatment of any individual patient. The same is true of the diffusible calcium determined by the method outlined.

Studies such as this will not rule out the possibility of disturbances of calcium metabolism in jaundice. The recent work of Minot and Cutler (1927–28) has stressed the importance of calcium in combating the toxic effect of carbon tetrachloride poisoning in the absence of changes in the calcium content of the serum. A similar relationship may be present in obstructive jaundice, but evidence of its presence is not to be shown in the adult dog or in man by changes in the amount or condition of the calcium in the serum.

SUMMARY

The normal range of variation of the calcium content of the serum and the changes produced by biliary obstruction have been determined in the dog and in man.

The mean calcium content of the serum of normal adult dogs was 10.77 ± 0.05 mgm. for each 100 cc., with a standard deviation of 0.78 mgm. Following experimental biliary obstruction the mean value was 10.16 ± 0.05, with a standard deviation of 0.72.

The mean calcium content of the serum of a control group of hospital patients was 9.96 ± 0.03 mgm. for each 100 cc., with a standard deviation of 0.69 mgm. In clinical cases of jaundice the mean value was 9.60 ± 0.05, with a standard deviation of 0.63. This reduction in the calcium content of the serum in the presence of jaundice is significant statistically, but it is probably not significant so far as the individual patient is concerned.

There was no significant disturbance in either the amount or proportion of diffusible calcium in the serum in the presence of jaundice.

There was no correlation between either the total or diffusible calcium content of the serum, and either the serum bilirubin or the delay in the coagulation time of the blood in the presence of jaundice.

Jaundice does not produce a clinically significant disturbance in either the quantity or condition of the calcium in the serum of adult dogs or hospital patients.

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CHEMICAL FACTORS IN VENTRICULAR FIBRILLATION[1]

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In an earlier paper (1) the writer described a method for the resuscitation of the heart in electric shock. As is known, the common cause of death in electric accidents is ventricular fibrillation. This remarkable condition, first described by Hoffa and Ludwig (2) in 1850, has stimulated many studies which have led to a number of theories to account for the phenomenon. These theories have been reviewed and attractively discussed by Garrey (3) and by Gallavardin (4). Attempts to account for the phenomenon, in the main, deal in physical concepts. The purpose of the present paper is to report results which suggest the possibility that chemical factors should likewise receive consideration. Thus it is conceivable, as originally suggested by Gotch (5), that the electric stimulus or whatever throws the ventricles into fibrillation, may produce an atomic disturbance of some protein-salt molecule such that an abnormal chemical millieu surrounds the muscle fibres with a consequent change in their behavior.

Support for an hypothesis of this character could be shown if experimental changes in the nutrient fluid entering the coronary arteries contributed to or resulted in the establishment of fibrillation. It is the purpose of the present paper to advance such supporting evidence.

The principle of the method for resuscitation from ventricular fibrillation, referred to above, consists of the use of two solutions: KCl 0.5 per cent in NaCl 0.5 per cent and $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent. These solutions are saturated with oxygen, warmed to body temperature and injected in sequence into the central end of the carotid artery under a pressure of 150 mm. Hg. The arterial blood pressure falls almost to zero as soon as fibrillation sets in. Consequently the sudden, forceful entry of the solutions is believed to carry them into the coronary vascular bed where they exert an influence on the cardiac musculature exactly comparable to that which they exert when perfused through the isolated heart. Hering (6) demonstrated the striking ability of a little strong potassium

[1] Published with the approval of the Physiological Committee of the Conference on Electric Shock.

chloride to stop ventricular fibrillation when introduced into the perfusion cannula of an isolated heart. In such a preparation the heart is at first completely inhibited and then, as the excess potassium is washed out by the normal perfusate, a regular, sequential cardiac rhythm is reëstablished.

The purpose of the first solution, therefore, is to stop the fibrillation. About thirteen cubic centimeters per kilo are required and with this 0.25 mgm. per cubic centimeter of heparin is injected to guard against coagulation. The cardiac rest thus produced will persist unless the excess potassium is removed. Accordingly an injection of the calcium solution is required, about twenty-five cubic centimeters per kilo. If the heart has been long in fibrillation (three to five minutes) resuscitation is favored by dividing the calcium injection to permit the introduction of one cubic centimeter of adrenalin chloride 1:1000.

In the course of the development of this technical procedure various modifications were tried for its improvement. These modifications suggested the idea which is the basis of this paper, namely, that at bottom fibrillation may depend upon or be associated with chemical changes in the tissue. This conception lurks in the background of two contributions to ventricular fibrillation but has, perhaps, not received deserved emphasis. Thus de Boer (7) found that single electric shocks would produce fibrillation in the exsanguinated frog heart at only one particular instant after the refractory phase; at any other time it was either ineffective or was followed by a simple extra systole and Porter (8) observed the onset of fibrillation following the anemia of coronary occlusion. Nevertheless prevailing views seem to stress physical in contrast to chemical processes in the maintenance if not in actual origination of the phenomenon under discussion.

Experimental. The following experiments fall into two groups. The first deals with the action of sodium bicarbonate and the second with the action of calcium chloride.

Action of bicarbonate. Although no study of the metabolism of the heart has been made during ventricular fibrillation, it was justifiably assumed that active metabolism continues with the production of acrid metabolites. Observation of the fibrillating heart *in situ* supports such an assumption. The fibrillation playing over the surfaces of the ventricles appears at first to be general and vigorous; it then slowly weakens and fades gradually away. If such a heart be held in the hand the same impression is received. The fibrillation dies down much as the normal beats die down when the circulation is stopped. The same thing is true of the isolated and perfused heart if the perfusion is cut off coincident with the establishment of fibrillation. If, on the contrary, the artificial circulation is maintained the fibrillation will continue with its initial vigor for at least two hours (experiment of Oct. 28, 1928) and the heart may then be brought back to an orderly sequential rhythm by introducing a little strong

potassium chloride into the perfusing cannula, followed by the normal balanced perfusate.

In consequence of these observations it seemed highly probable that the addition of sodium bicarbonate to the potassium solution would be of benefit. But the contrary proved to be the case. With the solutions approximately as recommended[2] failure to recover the heart was rare; when bicarbonate was administered with the potassium failure to recover became the rule. The evidence for the first part of this statement is given elsewhere but it may be here stated that in twenty-one consecutive experiments, variously modified *but without the use of bicarbonate*, there was one doubtful result and one outstanding failure. The evidence for the latter part of the statement is found in the following protocols.

Nine convincing experiments were performed but only three typical ones are here reported. In all of them the thorax was opened to permit accurate observation of the heart. The ventricles were fibrillated by the direct application of a tetanic stimulus. The solutions, unless otherwise noted, were saturated with oxygen and warmed to body temperature. Injection was made through a cannula in the right carotid under a pressure of 150 mm. Hg.

March 8, 1928. Female, 4.5 k. Morphia, veronal and ether. Fibrillated and left in fibrillation 1 minute, 30 seconds.

Inject 45 cc. KCl 0.5 per cent in NaCl 0.9 per cent. Heart rests.

Wait 1 minute and inject 120 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 1 cc. adrenalin 1:1000. In 3 minutes, 50 seconds after fibrillation heart beating normally.

Fibrillated and left in fibrillation 1 minute, 15 seconds.

Inject 45 cc. $NaHCO_3$ 0.02 per cent in KCl 1.0 per cent. Heart rests.

Wait 1 minute and inject 130 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 1.25 cc. adrenalin 1:1000. This is followed by a few feeble beats which shortly turn into fibrillation.

Inject 40 cc. KCl 0.5 per cent in NaCl 0.9 per cent. Heart rests.

Wait 30 seconds and inject 110 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 1.30 cc. adrenalin 1:1000. The heart makes a perfect recovery 8 minutes, 30 seconds after the original fibrillation, followed with spontaneous respiratory movements.

Animal sacrificed.

May 21, 1928. Female, 3.7 k. Morphia, veronal and ether. In this experiment the potassium-bicarbonate solution (containing 0.08 per cent $NaHCO_3$) showed a reaction of pH 8.2 by colorimetric determination. The other solutions were adjusted to the same pH by the addition of NaOH.

Fibrillated and left in fibrillation 1 minute.

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[2] It should be pointed out that all the experiments (performed upon dogs) reported in this paper were subjected to various modifications in the search for the most efficacious method of resuscitation. It follows, therefore, that they are not correlated as to details but only those are here presented which give definite evidence on the points under consideration.

Inject 30 cc. KCl 0.5 per cent, $NaHCO_3$ 0.08 per cent in NaCl 0.9 per cent. Heart rests.

Wait 1 minute and inject 65 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent. Heart began to fibrillate but recovered a normal beat in a short time.

Fibrillated and left in fibrillation 1 minute.

Inject 30 cc. KCl 0.5 per cent, $NaHCO_3$ 0.08 per cent in NaCl 0.9 per cent. Heart rests.

Wait 1 minute and inject 80 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent.

Fibrillation returns and continues.

Inject 30 cc. KCl 0.5 per cent in NaCl 0.9 per cent. Heart rests.

Wait 1 minute and inject 90 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 1 cc. adrenalin 1:1000.

Heart makes a perfect recovery.

Fibrillated and left in fibrillation 1 minute.

Inject 30 cc. KCl 0.5 per cent $NaHCO_3$ 0.08 per cent in NaCl 0.9 per cent. Heart rests.

Wait 1 minute and inject 100 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent. This is followed by a few feeble beats, then fibrillation returns.

Animal sacrificed.

December 17, 1928. Female, 6.4 k. Morphia, veronal and ether. In this experiment no oxygen was used and the solutions were injected at room temperature.

Fibrillated and left in fibrillation 30 seconds.

Thirty seconds. Inject 29 cc. KCl 0.5 per cent in NaCl 0.9 per cent. Heart rests.

One minute, thirty seconds. Inject 100 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 2.5 cc. adrenalin 1:5000. Heart makes perfect recovery.

Six minutes. Fibrillated.

Six minutes, thirty seconds. Inject 30 cc. KCl 0.5 per cent, $NaHCO_3$ 0.1 per cent in NaCl 0.9 per cent. Heart rests.

Seven minutes, thirty seconds. Inject 100 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent with 2.5 per cent adrenalin 1:5000.

Nine minutes, twenty seconds. Extremely fast beat but it is not fibrillation.

Sixteen minutes. Fibrillated and left in fibrillation 30 seconds.

Sixteen minutes, thirty seconds. Inject 30 cc. KCl 0.5 per cent $NaHCO_3$ 0.1 per cent in NaCl 0.9 per cent. Heart rests.

Seventeen minutes, thirty seconds. Inject 100 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent. Feeble beat in 31 seconds.

Eighteen minutes, thirty seconds. Inject 2 cc. adrenalin 1:5000 with a little calcium solution. Same fast beat but it is not fibrillation.

The careful reading of the above protocols will give a clear impression that the use of sodium bicarbonate conduces to a return of fibrillation. The results suggest that this is a border-line effect because in one test in the experiment of May 21, 1928, the fibrillation which returned was not permanent and in the experiment of December 17, 1928, in which the solutions were used at room temperature and without being saturated with oxygen, the effect of the bicarbonate, as seen in the subsequent behavior of the heart, was a violent tachycardia which did not quite pass into fibrillation. It is possible that failure to develop fibrillation in this heart was associated with the use of cold solutions.

In one of these experiments, that of May 21, 1928, the solutions were adjusted to the same pH. The outcome, although not wholly definite, suggests that the bicarbonate effect is unrelated to the hydrogen ion concentration determined by the use of this substance. This point is of both practical and theoretical importance and it was investigated in four additional experiments.

These additional experiments were performed on the isolated and perfused heart, using Locke's solution containing sodium bicarbonate on the one hand and on the other hand an identical solution, except that the bicarbonate was omitted, brought to the same pH by the addition of sodium hydroxide. The strength of current required to throw the ventricles into fibrillation when these two solutions were perfused was determined using the position of the secondary coil as an index. The following protocol will suffice to show the character of result obtained:

May 14, 1928. Male, 2.8 k. Morphia, veronal and ether. Bicarbonate solution: NaCl 0.9, $CaCl_2$ 0.023, KCl 0.025, dextrose 0.1, $NaHCO_3$ 0.08; pH 8.3. Hydroxide solution: identical throughout except that bicarbonate was omitted; the pH was brought to 8.3 by the addition of sodium hydroxide. The reaction of the solutions had not changed appreciably at the end of the experiment. They were oxygenated, maintained at 34.35°C. and perfused at 60 mm. Hg.

Procedure: With the heart fed with one or the other solution the secondary coil was moved up one quarter of a centimeter at a time until 5 seconds' application of the stimulus established permanent fibrillation. KCl 1 per cent was introduced into the perfusion cannula in amount sufficient to rest the heart. The solutions were then changed and the requisite stimulus again determined.

SOLUTION	TIME PERFUSED	FIBRILLATION WITH SECONDARY COIL AT
	minutes	
Hydroxide		8.5
Bicarbonate	2	9.5
Bicarbonate	2	9.25
Hydroxide	2	8.0
Hydroxide	2	8.0
Bicarbonate	2	Spontaneous fibrillation
Hydroxide		4.5
Bicarbonate	4	8.0

After the last observation the heart was put on the hydroxide solution for ten minutes but the ventricular contractions were no longer vigorous and the experiment was discontinued.

The above method of testing the action of bicarbonate in its relation to ventricular fibrillation is not wholly satisfactory but, in connection with the experiments already reported, it is strongly suggestive of some relation-

ship. Its presence in the perfusate certainly increases the irritability of the heart and in one instance it appeared to be responsible for the development of spontaneous fibrillation.

Action of calcium chloride. In the resuscitation procedure described elsewhere the calcium solution was thought to have a twofold function: to wash the excess potassium out of the coronary bed and to antagonize the potassium effect. Chiefly to accomplish the latter action and to avoid flooding the vascular system with a plethora of salt solution, a stronger solution of calcium was tried in the hope that a lesser volume would suffice. The amount of calcium was increased from 0.023 to 0.1 per cent. The outcome was not satisfactory; while some recoveries were had they were interspersed with a greater number of failures.

To check this difficulty resort was again taken to the isolated heart. Five experiments were performed, all in substantial agreement in showing that this increase of calcium conduces to fibrillation. The general procedure was designed to simulate conditions which obtain in the intact animal, thus: 1, the heart was isolated on Locke's solution; 2, fibrillated with direct application of a tetanizing stimulus and the perfusion stopped; 3, waited a fixed period and perfused with KCl 0.5 per cent in NaCl 0.5 per cent until the heart rested (in turning to another solution the proper temperature was secured by wastage through a side-tube close to the cannula); 4, waited a fixed period and perfused with the calcium solution. The following experiment is typical:

March 26, 1929. Female, 6 k. Morphia and ether. Heart isolated on oxygenated Locke's solution, 32–33°C., 60 mm. Hg.

1. Fibrillated, stopped perfusion and waited 5 minutes.

Perfused with KCl 0.5 per cent in NaCl 0.5 per cent until heart rested, stopped perfusion and waited 1 minute.

Perfused with $CaCl_2$ 0.119 per cent in NaCl 0.9 per cent, injecting therewith 2 cc. adrenalin 1:2000.

Fifty-five seconds after the last perfusion was begun the heart gave one beat and fell into fibrillation. Changed to perfusion with Locke.

2. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution, injecting therewith 2 cc. adrenalin 1:2000.

Heart beat normally for 2 to 3 minutes then fell into fibrillation spontaneously.

3. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution.

Normal beats.

4. Fibrillated, stopped perfusion and waited 5 minutes.

Perfused with potassium solution until heart rested, stopped perfusion and waited 1 minute.

Perfused with Locke's solution.

Normal beats.

5. Fibrillated, stopped perfusion and waited 5 minutes.

Perfused with potassium solution until heart rested, stopped perfusion and waited 1 minute.

Perfused with calcium solution.

Few normal beats followed by fibrillation. Changed to perfusion with Locke.

6. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution, injecting therewith 2 cc. adrenalin 1:2000.

Normal beats.

7. Fibrillated, stopped perfusion and waited 2 minutes.

Perfused with potassium solution until heart rested, stopped perfusion and waited 1 minute.

Perfused with calcium solution.

Few normal beats followed by fibrillation. Changed to perfusion with Locke.

8. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution.

Normal beats.

9. Changed suddenly to perfusion with calcium solution. After a brief period the heart went into fibrillation spontaneously. Changed to perfusion with Locke.

10. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution.

Normal beats.

11. Introduced 6 cc. adrenalin 1:2000 into cannula. Strengthened beats but did not cause fibrillation.

12. Fibrillated, stopped perfusion and waited 2 minutes.

Perfused with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with calcium solution.

Fibrillation returned. Changed to perfusion with Locke.

13. Still fibrillating. Changed to perfusion with potassium solution until heart rested; stopped perfusion and waited 1 minute.

Perfused with Locke's solution.

Normal beats.

14. Introduced 2 cc. adrenalin 1:1000 into cannula. Strengthened beats but did not cause fibrillation.

15. Heart beating nicely 30 minutes later. Experiment stopped.

This protocol shows that $CaCl_2$ 0.119 per cent in NaCl 0.9 per cent when used to wash out the potassium conduces to a return of a preëxisting fibrillation. This is not the case if Locke's solution is used (one exception, had with the coincident use of adrenalin). Furthermore, in one instance, the sudden transition from Locke to this calcium solution brought about spontaneous fibrillation.

In a subsequent experiment (April 1, 1929) a similar sudden transition from Locke to the same calcium solution resulted in spontaneous fibrillation in each of three trials. Incidentally it may be noted that in this experiment the introduction of 3 cc. adrenalin 1:1000 into the cannula, the heart being perfused with Locke's solution, did not cause fibrillation.

In the experiment of April 3, 1929, two similar trials with the strong calcium resulted in a violently fast heart which did not, however, pass into fibrillation.

The last protocol to be presented, that of June 4, 1929, is of interest because it shows that the combination of the usually employed amount of bicarbonate with an excess of calcium chloride is more conducive to fibrillation than is the excess of calcium alone.

June 4, 1929. Female 3 k. Morphia and ether. Calcium solution: $CaCl_2$ 0.15, NaCl 0.9 per cent; Locke's solution: NaCl 0.9, $CaCl_2$ 0.023, dextrose 0.1, $NaHCO_3$ 0.02 per cent; bicarbonate-calcium solution: same as last except $CaCl_2$ increased to 0.15 per cent.

The heart was isolated on Locke's solution at 31°C., and 60 mm. Hg.

1. Changed suddenly to calcium solution.

After 2 minutes, 55 seconds, injected 1 cc. adrenalin 1:1000.

After 4 minutes heart in fibrillation.

Changed back to perfusion with Locke. The heart returned to a normal beat spontaneously.[3]

2. Changed suddenly to bicarbonate-calcium solution. After 2 minutes, 55 seconds the ventricles went into fibrillation.

Changed back to perfusion with Locke; the fibrillation continued until four doses of KCl 2 per cent, of 2 cc. each, had been introduced into the cannula.

3. Changed suddenly to calcium solution.

Fibrillation had not developed in 4 minutes. Changed back to perfusion with Locke.

4. Changed suddenly to bicarbonate-calcium solution

After 2 minutes injected 1 cc. adrenalin 1:1000.

After 3 minutes injected 1 cc. adrenalin 1:1000.

After 4 minutes heart in fibrillation.

Changed back to Locke and recovered normal beat by use of potassium chloride.

5. Changed suddenly to bicarbonate-calcium solution.

After about 4 minutes heart in fibrillation.

6. Experiment discontinued.

SUMMARY. Suggestive evidence has been presented in the foregoing pages in support of the idea that chemical factors may play an important part in the induction of ventricular fibrillation.

Sodium bicarbonate, irrespective of its buffer action, exerts an influence on the perfused dog heart such that a lighter stimulus is required to throw it into fibrillation; it may also induce spontaneous fibrillation if present in a perfusate which is suddenly substituted for a perfusate otherwise similar except for the absence of the bicarbonate. If used in conjunction with potassium chloride and calcium chloride in efforts to resuscitate a fibrillating heart *in situ* it lessens the chance of a successful recovery.

Calcium chloride, similarly, influences the dog heart with respect to

[3] This is the only instance in a very large number of observations in which such spontaneous recovery occurred.

fibrillation. If present in two perfusates prepared for the isolated heart in amounts of 0.023 per cent and 0.15 per cent the sudden transition from the weaker to the stronger solution will usually throw the ventricles into fibrillation. The presence of calcium chloride stronger than 0.023 per cent in the solution used in efforts to resuscitate a fibrillating heart *in situ*, likewise lessens the chance of a successful recovery.

CONCLUSIONS

Chemical factors bear a more direct relationship to ventricular fibrillation than has heretofore been recognized. Instances are presented to show that sodium bicarbonate and calcium chloride may conduce to the induction of ventricular fibrillation.

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THE EFFECT OF DESICCATED SPLEEN AND SPLENECTOMY ON SERUM CALCIUM IN NORMAL AND PARATHYRODECTOMIZED DOGS

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It is well known that completely thyro-parathyroidectomized dogs treated by any of the several methods known to prevent or alleviate tetany during a period of 30 to 40 days will recover in a certain percentage of cases and show no further tetany. By what mechanism these animals recover and live without further treatment may be only postulated. The existing views that are given to explain why the animals recover are based upon the various theories relating to the etiology of parathyroid tetany. It has been suggested by some who believe that tetany is due to a toxin, that recovery results when antibodies are formed making the body immune to the existing toxins.

Dragstedt and co-workers (1) believe that the parathyroid glands form part of a detoxicating system of the body and, upon removal, a toxemia results producing tetany. They believe that the liver and endothelial system play some rôle in enabling dogs to recover from tetany. The findings of Blumenstock et al (2) (3) on Eck fistula thyroparathyroidectomized dogs do not support the view that the liver is a detoxifying agent.

Those who support the calcium theory and those who have expressed any opinion as to the mechanism of recovery form parathyroid tetany believe that either accessory parathyroids hypertrophy, or that some other organ takes over the function of the parathyroids, or that the body becomes adjusted to a lower level of calcium.

It has been the purpose of this work to find whether the spleen may assume the function of the parathyroids in recovered para-thyroidectomized animals. Hall and Ablahadian (4) found that splenic extract caused an increase in blood calcium with the apex of the curve occurring in twenty-four hours. They also reported that the blood calcium dropped following splenectomy. Krumbhaar (5) (6) cited a possible relationship between it and the parathyroid glands. He stated that unfinished work of his own tended to confirm the results of Hall and Ablahadian to a certain extent.

Hammett (7) in studying the relations between the spleen and parathy-

roids stated that the former is specifically affected by parathyroid deficiency with a tendency toward hypertrophy. These findings would seem to warrant the study of the effect of splenectomy on recovered parathyroidectomized dogs. If the spleen is concerned with calcium metabolism as has been suggested, then splenectomy in recovered thyro-parathyroidectomized dogs should at least cause a drop in serum calcium and possibly result in tetany.

The spleen was removed from eight normal dogs and serum calcium estimations were made both before and subsequent to operation. In this group, the average drop in serum calcium in seven out of the eight animals was 2 to 3 mgm. from the average normal level. As a control for this experiment, five normal dogs were subjected to laparotomy or to other operations used in physiological research. These operations produced no change in serum calcium.

In a third group, six splenectomized dogs with serum calcium below normal were given 50 grains of desiccated spleen (Armour) daily for a period of 5 to 8 days. The serum calcium increased on an average of 1.5 to 2 mgm. per 100 cc. of blood.

In a fourth group of three parathyroidectomized dogs, 40 grains of desiccated spleen were ineffective in raising serum calcium or preventing the onset of tetany.

In a fifth group of five animals, the parathyroids were removed and after recovery from tetany, the dogs were splenectomized. Three of these animals had been treated with calcium and had recovered, but the serum calcium never returned to normal. Splenectomy further decreased this level on an average of 2 to 2.5 mgm.; two animals developed tetany and one died. The other two parathyroidectomized dogs were given cod liver oil, and after 30 to 40 days serum calcium returned to normal with no further appearance of tetany. Although splenectomy in these two animals lowered the serum calcium, no symptoms of tetany developed.

Estimations of serum calcium were made by Collip and Clark's modification of the Kramer and Tisdall method.

Discussion. From the preceding results it would appear that some dependent relation exists between the serum calcium and the parathyroids and spleen.

The fact that serum calcium dropped following splenectomy and showed no change in other laparotomies points to a specific action of the spleen on calcium metabolism.

The occurrence of tetany in recovered thyro-parathyroidectomized animals following splenectomy might be interpreted to mean that the spleen had to some extent taken over the function of the parathyroids. Whether the removal of some other organ such as the liver would also produce tetany cannot be stated.

CONCLUSION

Splenectomy in normal and recovered thyro-parathyroidectomized animals produced a fall in serum calcium. Desiccated spleen caused an elevation in serum calcium in splenectomized animals but not in parathyroidectomized animals. There seems to be an interrelationship between serum calcium, the spleen and the parathyroids, but it cannot be said that the spleen compensates for the loss of the parathyroids.

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EFFECT OF ULTRAVIOLET IRRADIATION ON THE MAGNESIUM CONTENT OF RATS RECEIVING REFLECTED SUNLIGHT AND A UNIFORM STOCK RATION[1]

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Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station

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Until very recent years it has been assumed that rickets was primarily caused by a calcium deficiency in the blood plasma. However, according to Gamble (1), the results of experimental rickets emphasize the fact that the outstanding feature of the disease is an incorrect metabolism of phosphorus rather than a deficiency of calcium.

It has been demonstrated by experiments with rats that rickets may be induced at will by any one of three different ways: 1, by unfavorable changes in the amounts and proportions of calcium and phosphorus supplied through the blood; 2, by the deficiency of the antirachitic vitamin; and 3, by the deficiency of ultraviolet irradiation.

Mellanby (2) observed that cereals apparently contain a substance that interferes with the proper calcification of bones.

Cattaneo (3) analyzed rickety bones and found that the calcium content was not much changed while the magnesium content was considerably increased.

Malcolm (4) has published data which show that the ingestion of soluble magnesium salts causes a loss of calcium in adult dogs and hinders the deposition of calcium in young, growing rats.

Forbes (5) suggests that the cause of "bran disease," "shorts disease" and "Miller's horse rickets" is due to an excessive proportion of magnesium to calcium in these food materials.

The principal purpose of the experiments described in this paper was to ascertain if a deficiency of ultraviolet irradiation was affecting, to any appreciable degree, the growth of our stock rats. Since obtaining the parent stock of Albino rats five years ago succeeding generations have been kept in a room from which the direct rays of the sun are excluded by shade trees during the summer time and after the leaves are off the trees in the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

autumn the sun shines into one of the two plain glass windows of the room for about an hour in the late afternoon, but does not reach the cages containing the rats which are in the back of the room and about 10 feet from the window. This condition eliminates the possibility of the rats receiving any beneficial effect of direct irradiation from the sun.

During the time in which our colony has been maintained, perhaps a thousand or more rats have been raised and no disease has been observed at any time among them. The older rats are eliminated from the colony from time to time as their age becomes conspicuous, otherwise no other than the ordinary attention to food, water and sanitation has been necessary. However, at different intervals it has been observed that the rats apparently do not thrive and reproduce as freely as at other times of the year, although the ration has remained practically constant.

The regular ration fed the stock rats contains the following ingredients:

	per cent
Whole wheat	25
Yellow corn	30
Rolled oats	30
Linseed oil meal	10
Alfalfa leaf meal	5

The ingredients are finely ground and the ration is available practically all the time through self-feeders attached to the cages. In addition the rats receive such peelings and table scraps as are available from a family of two persons and occasionally a small quantity of whole milk.

The analysis of the ration for the stock rats is as follows, in percentage of the moisture-free material:

Crude ash	2.55
Copper (Cu)	0.00074
Manganese (Mn)	0.0029
Iron (Fe)	0.022
Zinc (Zn)	0.0028
Calcium (Ca)	0.164
Magnesium (Mg)	0.173
Phosphorus (P)	0.422
Sulfur (S)	0.245
Chlorine (Cl)	0.900
Potassium (K)	0.489
Sodium (Na)	0.096
Fat	4.26
Crude Fiber	4.37
Nitrogen (N)	3.31
Protein (N. 6.25)	20.70

With two normal mother-rats which were kept in separate cages and their litters of 10 pups each the following experiments were carried out to

ascertain the effect of irradiation with an ultra-violet lamp on one-half of the number of rats in each of the litters.

On the day of their birth the tails of 10 of the rat pups, 5 in each of the litters, were clipped for the purpose of future identification and the clipped and unclipped pups in each cage weighed separately but collectively. The marked pups in each cage were irradiated for about 3 minutes every other day for about three weeks. After this time they were irradiated for five minutes daily during the remaining 34 days of the experiment. The ten unmarked rat pups remained in the cages with their mothers as did those that were marked except for the time the latter were receiving irradiation, and each lot received the same kind of food during the time of the experiment.

TABLE 1

Weights of rats during the experiment

	CAGE 1		CAGE 2	
	Weights of 5 rats irradiated	Weights of 5 rats not irradiated	Weights of 5 rats irradiated	Weights of 5 rats not irradiated
	grams	*grams*	*grams*	*grams*
June 25, 1929	28.5	28.0	28.0	27.0
June 29, 1929	40.0	40.5	40.0	39.5
July 6, 1929	64.0	64.0	63.0	62.0
July 13, 1929	91.0	91.0	90.0	90.0
July 20, 1929	143.0	145.0	142.0	138.0
July 27, 1929	196.0	198.0	192.0	180.0
August 3, 1929	257.0	244.0	237.0	219.0
August 10, 1929	311.0	301.0	322.0	273.0
August 17, 1929	379.0	359.0	406.0	338.0
August 19, 1929	386.0	363.0	418.0	346.0
Total gain	357.5	335.0	390.0	319.0
Increased gain by irradiation	22.5		71.0	

Table 1 contains the weights of the irradiated and the non-inrradiated rats during the time of the experiment.

At the conclusion of the experiment all the rats were chloroformed, the intestinal tracts removed and the carcases dried at 100°C. for chemical analysis. Three of the irradiated and three of the non-irradiated rats in each cage were ashed and analyzed separately to determine if any material difference in the mineral content of the two lots of rats had been effected as a result of the irradiation. Furthermore, the leg bones of the remaining four rats (two irradiated and two not irradiated) were dissected out, ashed and analyzed for calcium, magnesium and phosphorus. The results of the analyses of the carcases and leg bones of the irradiated and non-irradiated rats, calculated in per cent of the moisture-free material, are contained in tables 2 and 3.

COMMENTS. The results contained in tables 1 and 2 show a decided increase in the live weight, the dry weight, the ash and certain mineral constituents of the irradiated rats in comparison with those that were not irradiated.

However, the most striking difference and one which is probably of most significance is the effect of irradiation on the magnesium content of the

TABLE 2

Analyses of the carcases of irradiated and non-irradiated rats, calculated as per cent of the moisture-free material

TREATMENT	CAGE 1		CAGE 2	
	Three rats irradiated	Three rats not irradiated	Three rats irradiated	Three rats not irradiated
Dry weight (grams)	51.7680	50.0680	56.5518	44.5200
Total ash (grams)	5.5892	5.3498	5.7633	4.9856
Crude ash	10.20	10.68	10.19	11.20
Copper (Cu)	0.0008	0.0009	0.0007	0.0008
Manganese (Mn)	0.0003	0.0002	0.0002	0.0002
Zinc (Zn)	0.0139	0.0128	0.0121	0.0125
Iron (Fe)	0.044	0.038	0.036	0.037
Calcium (Ca)	2.70	2.72	2.55	2.82
Magnesium (Mg)	0.086	0.142	0.075	0.133
Phosphorus (P)	2.12	2.12	2.05	2.15
Potassium (K)	1.06	1.08	1.05	1.16
Sodium (Na)	0.753	0.756	0.914	0.577

TABLE 3

Analyses of the leg bones of irradiated and non-irradiated rats, calculated as per cent of the moisture-free material

TREATMENT	CAGE 1		CAGE 2	
	Two rats irradiated	Two rats not irradiated	Two rats irradiated	Two rats not irradiated
Calcium (Ca)	15.14	17.55	15.07	15.88
Magnesium (Mg)	0.194	0.307	0.165	0.274
Phosphorus (P)	9.42	10.96	9.54	10.10
Ash	44.02	50.09	43.14	46.16

two lots of rats. The amount of magnesium contained in the carcases of the irradiated rats is a little more than one-half as much as in those that were not irradiated. Since all the rats received the same ration which was composed largely of grains relatively rich in magnesium it appears that one of the effects of irradiation was to enhance magnesium elimination whereas in the non-irradiated rats there was an accumulation of magnesium

which probably was a cause of their retarded growth. Although the non-irradiated rats did not attain a state fully characteristic of rickets it is believed they were so inclined.

The calcium content of the leg bones of the irradiated rats is appreciably less than that of the non-irradiated rats, whereas the magnesium content is only a little more than one-half as much as in the bones of the rats that were not irradiated. The phosphorus content is considerably less than that in the bones of the rats that were not irradiated.

The results obtained in these experiments tend to confirm the ideas expressed by Mellanby, Cattaneo, Malcolm and Forbes, that magnesium is associated with rickety bones and may be a more important factor in the production of rickets than has hitherto been supposed. It is also apparent that one of the beneficial effects of ultraviolet irradiation is the elimination of an excess of magnesium, thereby maintaining a proper balance between calcium, magnesium and phosphorus in the metabolism of animals.

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THE EFFECT OF PROLONGED ACTIVITY ON THE IRRITABILITY OF MEDULLATED NERVE

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Attention has recently been again directed to the question of fatiguability or equilibration in peripheral nerves. It seems now well established that prolonged tetanization of a nerve leads to a diminution of the amplitude of the action potential (Thörner, 1909; Tigerstedt, 1912; Forbes and Rice, 1929). Tigerstedt likewise finds a prolongation of potential, though this is questioned by Heinbecker (1929). There is also a lengthening of the refractory period (Field and Brücke, 1926; Gerard and Forbes, 1928) and a lowering of the heat production and oxygen consumption per impulse (Gerard, 1927).

Little work has been done on the effect of prolonged stimulation on the excitability of nerve. Local "stimulation fatigue" is a well known phenomenon (Gotch, 1910), but probably is not to be considered as a manifestation of fatigue in the proper sense of the term. Thörner (1909) found that after tetanization for several minutes the threshold for faradic stimuli is raised. This effect is increased during asphyxia (Heinbecker, 1929). We know of only one reported observation (L. and M. Lapicque, 1919) on the excitability of fatigued nerve made by chronaximetric methods. Such a study we wish to report.

METHOD. A sciatic-gastrocnemius preparation of the green frog was placed in a paraffin moist chamber. The nerve was laid over two pairs of electrodes, A and B (see fig. 1) distant about 2 and 5 centimeters, respectively, from the muscle. The "A" electrodes were of silver-silver chloride. They were connected through a Lapicque shunt with a variable mica condenser which was charged from a Boulitte potential divider. The "B" electrodes, of plain silver wire, were connected to the secondary of a Harvard inductorium.

The rheobase and chronaxie of the resting nerve were first determined by stimulation at *A*, using the minimal visible twitch of the muscle as the criterion of excitation. After a series of such readings, made at intervals of four or five minutes, the nerve was blocked by cold (at *C*) about half a centimeter from the muscle. When completely blocked, the nerve was tetanized from the "B" electrodes for a period varying between 4 and 17

minutes. The tetanizing stimuli, at a frequency of 100 shocks per second, were made somewhat supermaximal for the resting nerve, to prevent local stimulation fatigue from rendering them ineffective. (With two volts in the primary circuit the secondary coil was usually set at 12 centimeters and turned 30° from the horizontal.) After a sufficient time the block was removed, tetanization being continued until the muscle began to contract. Stimulation at *B* was then stopped, and the excitability tested again at *A*.

With the procedure as outlined, there are several possible sources of error in making and interpreting the measurements. Among the most obvious are the following:

1. The "fatigued" nerve always had time for a certain degree of recovery before chronaximetric readings could be finished. Measurement of the rheobase required 25 to 35 seconds after tetanization was stopped, and about 15 seconds more elapsed before the chronaxie was determined. It is quite possible that significant changes in excitability escaped observation because of the intervening period of rest (see, e.g., Forbes and Rice, 1929). Some fatigue effects, however, have been shown to last for a comparatively long time. Field and Brücke (1926) found the lengthening of the refractory period to persist in some instances for as long as ten minutes. Gerard (1927) found that the extra heat production and oxygen consumption and the potential changes of activity are influenced by a previous activity, even though several minutes of rest have intervened. It has proved possible here also to detect changes in excitability after half a minute of rest. Incidentally, the progressive fall of rheobase, between the determination of rheobase and chronaxie, may have masked a slight rise of the latter.

2. While important to make the readings as early in the rest period as possible, it was equally important not to make them until conduction had returned in all the nerve fibers. The production of a visible muscle twitch means that a certain minimum number of nerve fibers must be excited. In a partially blocked nerve, unless the conducting and non-conducting fibers are arranged in a very improbable way with respect to the electrodes, it should require a relatively stronger stimulus, i.e., a higher rheobase, to reach the required number of conducting fibers.

3. To avoid fatigue effects on the muscle, it was desirable to stop the tetanizing stimulus as soon as conduction returned in any fibers of the nerve.

It will be evident that in seeking to avoid one of the above difficulties there was danger of running into another. There is apparently no known blocking agent which will permit all of them to be perfectly met. The cold block seemed to be the best available. It is rapidly reversible, and under the conditions of these experiments conduction seems to be restored nearly simultaneously in all the fibers. There is probably as little danger

of damage to the nerve as with any other method. Brodie and Halliburton (1902) maintained a cold block for several hours with apparently complete return of conductivity.

We also carried out a few experiments using galvanic block. The results were similar to those with cold block.

Our original intention was to record single nerve action currents with the Downing galvanometer, and this would have eliminated all the complications introduced by a block. One successful experiment had been carried out when trouble with the galvanometer caused us to seek another method. The results obtained in this case were in harmony with the others. With a less sensitive galvanometer available, the summed electric responses to a tetanus could be easily observed and used as the index of successful excitation of the nerve. The threshold intensity was determined for a tetanus applied at one pair of electrodes, the nerve then tetanized with maximal stimuli at a second pair and the threshold again determined at the first. The threshold was expressed as the maximum resistance in the primary of a coreless coil, or the greatest angle between the primary and secondary coils (12 cm. apart), at which a deflection could be observed. Since maximal deflections were of the order of 100 mm. and readings were easily made to 1 mm., the threshold could be determined with considerable precision.

The arrangement used for blocking was suggested to us by Dr. J. P Quigley. It consisted simply of a tin can, of about one liter capacity, with a small silver rod (diameter 1.5 mm.) soldered to its outer surface and projecting into the moist chamber (fig. 1). The nerve was wound once around this rod, 12 to 15 mm. from the can. On putting a freezing mixture at $-10°$ to $-12°$C. into the can, the nerve could be completely blocked within 5 minutes. Ordinarily, however, the temperature of the freezing mixture was gradually reduced, so that the nerve was cooled over a period of 10 to 20 minutes before a complete block developed. On replacing the freezing mixture with water at 25°, conductivity returned in 20 to 40 seconds.

In order to prevent the entire nerve from being cooled, the moist chamber was divided into two compartments, as shown in figure 1. The nerve passed through a slot in the partition and was sealed in with vaseline. Except for this slot and the side walls, the compartment containing the electrodes was lined with sheet copper, thinly coated with paraffin. From the floor a thick bar of the same metal (not shown in the figure) passed through the wall and dipped into a dish of water at room temperature. Conduction along this metal kept the air temperature in the compartment within 2°C. of that outside. All experiments were carried out at room temperature, 22–24°C.

In our early experiments the nerve was simply laid across the bare metal rod. It proved difficult to bring about a complete block in this manner.

Using single maximal shocks, from the "B" electrodes, we found sometimes an interval of ten minutes or more between the first falling off of the contraction and its final extinction. In fact the nerve was occasionally found stiffly frozen to the rod, with the muscle still responding to stimulation at *A* or *B*. Freezing to the rod was prevented by lightly coating the metal and the nearby portion of the nerve with vaseline. Winding the nerve around the rod permitted a much more rapid production or removal of the block.

Occasionally a nerve on being warmed again failed to regain its conductivity and had to be discarded. It was noted in such instances that conduction usually returned if the preparation was soaked in Ringer's solution for a few minutes, or even if the previously cooled region was washed with Ringer *in situ*. Tait (1908) mentions similar experiences. This delay in recovery became more rare as we learned the approximate temperature at which a block might be expected and took care not to go below it.

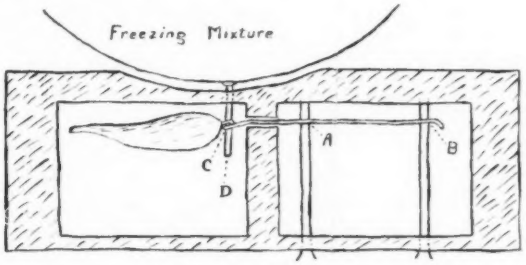

Fig. 1. Diagram showing arrangement of muscle-nerve preparation for stimulation and for blocking. *A*, electrodes for testing excitability. *B*, tetanizing electrodes *C*, point at which nerve was blocked. *D*, silver rod conducting to freezing mixture.

Results. Following tetanization and removal of the block, there was found quite constantly an elevation of the rheobase, with a subsequent return to, or toward, the original or resting value. The chronaxie remained practically unchanged, though sometimes it was shortened. The record of one experiment is shown in table 1.

Using double the original rheobase, rather than the increased one, the "chronaxie" of course shows an increase following tetanization. For example: before tetanization R = 0.68, C = 0.26; after tetanization R = 0.77, C = 0.28, but for the original R, C = 0.33.

As a rule, however, the rise in rheobase was relatively smaller than in the experiment just cited, and the readings of chronaxie were not always so uniform. The record of another experiment follows (table 2).

Altogether 25 experiments were performed with similar results. The rheobase showed an average increase of 27 per cent, and required 12

minutes (the duration of the delayed heat production) to return to normal. For longer tetanization the rise of rheobase was greater than for short. Thus an average tetanization of 4 minutes increased the rheobase 22 per

TABLE 1

July 6, 1929. Green frog, sciatic-gastrocnemius. Dissection finished at 10:45, preparation mounted at 11:00

TIME	RHEOBASE	CHRONAXIE	REMARKS
	volts	σ	
11:09	0.64	0.26	
11:15	0.64	0.26	
11:20	0.64	0.30	
11:25	0.64	0.30	
11:27			Freezing mixture applied
11:30	0.64	0.30	
11:33			Nerve blocked. Tetanized 9 minutes, 30 seconds
11:43	0.94	0.30	Readings completed 42 seconds after rest began
11:44	0.84	0.30	
11:45	0.75	0.30	
11:50	0.68	0.31	
11:54	0.64	0.30	
11:59	0.64	0.30	

TABLE 2

July 8, 1929. Green frog, sciatic gastrocnemius. Dissection finished at 9:40, preparation mounted at 9:55

TIME	RHEOBASE	CHRONAXIE	REMARKS
	volts	σ	
10:04	0.58	0.30	
10:10	0.55	0.32	
10:16	0.55	0.30	
10:20	0.55	0.31	
10:22			Freezing mixture applied
10:25	0.55	0.32	
10:30	0.55	0.33	
10:32			Nerve blocked. Tetanized 7 minutes 20 seconds
10:40	0.68	0.30	Readings finished in 45 seconds after rest began
10:41	0.61	0.33	
10:44	0.58	0.35	
10:46	0.55	0.32	
10:50	0.55	0.33	

cent, one of 13 minutes increased it 30 per cent. L. and M. Lapicque (1919) report an experiment on the toad in which the nerve rheobase was increased to over 15 times its resting value by 200 single stimuli and required half

an hour to return to normal, the chronaxie meanwhile remaining unchanged. We have never seen any such marked effect and suspect that their results were complicated by changes in the muscle.

As stated above, the elevation of rheobase (with little change in chronaxie) might be due to inactivity of the most irritable fibres or of some of those most accessible to the electrodes. That the unequal rate of recovery in different fibers is not the basis of the increased rheobase is shown by control experiments.

The duration of the partial block in the recovering nerve was determined in the following simple way. The nerve was blocked by cold and then warmed, exactly as described, but during the recovery period it was stimulated from *B*, with maximal stimuli, at intervals of 5 seconds. Kymographic records were made of the height of the muscle contractions. The

TABLE 3

July 16, 1928. Green frog, sciatic gastrocnemius

TIME SINCE DISSECTION	RHEOBASE	CHRONAXIE	REMARKS
minutes	*volts*	σ	
6	0.20	0.40	
8	0.20	0.42	
10	0.20	0.37	
10.5–11.5			Ascending galvanic current applied between
12	0.20	0.37	A electrodes and muscle
14	0.19	0.46	
16	0.20	0.39	
16–17			Descending galvanic current
17.5	0.20	0.40	
19	0.20	0.40	
19.5–20.5			Descending galvanic current plus tetaniza-
21	0.24	0.40	tion at B electrodes. Block complete
23	0.20	0.42	during the minute

interval between the earliest weak contraction and the first maximal one never exceeded 25 seconds. This period is too short to account for an elevation of the rheobase persisting for several minutes.

Experiments were also performed in which the nerve was blocked but not tetanized. This was done both with single and with double preparations. In the latter case the control nerve was simply blocked, while the opposite nerve was blocked and tetanized, chronaxie readings being made on both. The two were placed in the same chamber, the control nerve nearer to the freezing mixture than the "fatigued" one. The latter showed the usual rise in rheobase after blocking, the control nerve did not.

The same control experiments apparently serve to rule out another possibility, namely, that the change in excitability was due to a spread of

the cooling from the blocked area to the region of the B electrodes. As a further precaution, however, we reversed the electrodes, tetanizing from *A* and taking chronaxie readings from *B*, 3 centimeters farther from the cold wire. The results were similar to those obtained with the original arrangement.

Entirely analogous results, using a galvanic block, are shown in another record made a year earlier, table 3.

The results of one experiment, using the nerve response as the index of excitation, are shown in figure 2. It will be noted that, following a period of tetanization, the threshold remains high for some minutes. Also,

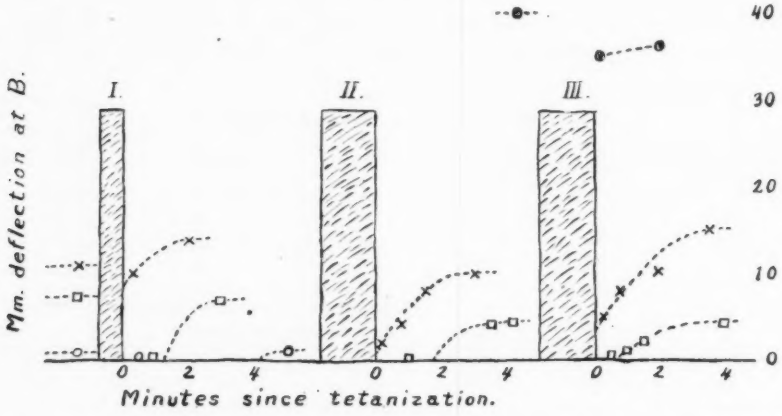

Fig. 2. Experiment July 17, 1929. Three periods of tetanization. I, at A electrodes, for two minutes. II, at B, for five minutes. III, at *A*, for five minutes. ○, response with secondary coil at 85°; □, at 83°, × at 80°, and ● at 0°, horizontal. Note that longer period of tetanization has greater effect, and that local stimulation (at *B*) has greater effect than at distance (local fatigue)

supraminimal stimuli elicit much feebler responses than after a rest. This indicates that any submaximal stimulus is able to affect fewer fibres, as should result if the threshold of each fibre rose. Thus if two fibres have thresholds of 1 and 2 at rest and 2 and 4 after tetanization, a stimulus of 3 would excite both at rest and only one when fatigued. The other possible basis for a decreased response to a supraminimal stimulus, decreased response per fibre, can be shown inadequate. The action potential produced by an over-threshold stimulus may be depressed after tetanization to 20 per cent or less of its resting value. If due to a similar depression in each fibre, then the response to a supramaximal stimulus exciting all fibres should fall off to the same extent. Actually no such fall is ever observed, a fall to 80 per cent being about the limit.

It is worth noting that a later effect of activity often appears as a decrease of threshold below the initial level. We have also often observed a fall in threshold, or "facilitation," even on repetition of single stimuli.

Discussion. A rise of rheobase with no chronaxie change (i.e., a change in voltage scale of the voltage-duration curve) has been interpreted by Lucas (1910), in terms of Hill's (1910) mathematical development as indicating an increase in the critical concentration of ions, at an irritable surface, required to produce excitation. A change in chronaxie, involving a change on the time scale of this curve, is similarly referred to alteration of ion mobility, such alteration depending largely on colloidal changes. Thus the change of irritability of a nerve produced by cooling—a lowered threshold for long enduring currents (lower rheobase) and an increased one for short currents (lengthened chronaxie)—described by Gotch and Macdonald (1896) is interpreted in terms of opposing effects on the ion mobility and on the required critical ion concentration.

Lapicque (1926) interprets the voltage-duration curve for electrical stimulation in terms of the capacity and resistance of the polarizable membrane of the axones. Excitation results when an imposed potential has produced a critical potential difference (difference in ion concentration) between two points at different distances from the membrane. An increased permeability of the membrane (lowered ohmic resistance) leads, on this theory, to an increased rheobase and decreased chronaxie. A change in external circuit resistance affects the rheobase only, one in membrane capacity, the chronaxie only. Cooling a nerve lowers the rheobase and increases the chronaxie as would a decreased membrane permeability.

The prevalent view of nerve function, and especially as analyzed by Gerard (1927, 1930), predicts, as a result of equilibration, a decreased membrane resistance and an increased threshold (in the sense of a rise of a critical potential or ion concentration required to set off the response). Thus, on either theory of excitation, a rise of rheobase is to be anticipated, and it is found. According to Lapicque's view, a simultaneous fall in chronaxie should be observed unless in some way balanced by an increase of capacity. The Hill-Lucas theory does not call for a change in time relations if the ion transfer is not altered. Actually, an unchanged chronaxie is the rule.

It must be noted, on the other hand, that a change in rheobase alone might represent merely altered conditions in the physical circuit. Of course no change in outside circuit, electrode contacts and the like need be considered in this type of experiment. It is conceivable, however, that increase in the number or mobility of ions in the intercellular fluid, acting as a low resistance shunt to the nerve membranes, would cause an increased rheobase. If this were the true explanation of this effect it would still constitute valuable evidence of marked metabolic changes produced by

activity and only slowly disappearing on rest. But it is an entirely gratuitous assumption that the conductivity of the tissue fluid, essentially Ringer's solution, can be increased a third or more as a result of the active metabolism of the nerve fibres.

Gerard and Wallen (1929) found a breakdown of phosphocreatin in nerve as a result of equilibration, and Nachmannsohn has shown in muscle that breakdown of phosphocreatin is associated with decreased irritability.

It is not our intention to enter here into a discussion of theories of electrical excitation. The significant finding is that as a result of and for minutes after a period of activity, or equilibration, the excitability, like the response, of nerve is definitely altered.

SUMMARY

The frog's sciatic was stimulated at a frequency of 100 per second for periods of 4 to 17 minutes, the attached gastrocnemius being protected during this time by cold or galvanic block. The excitability of the motor fibers was then tested, at another point on the nerve, by the chronaximetric method. Controls showed that the block itself did not affect the excitation. In some experiments response was measured in terms of nerve action currents and no block was used.

1. Even after thirty seconds of recovery, the rheobase is found on the average 27 per cent higher than that of resting nerve, and several minutes are required for the return to normal.

2. The chronaxie, under the same conditions, shows no change or a slight fall.

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SOME OBSERVATIONS ON THE BLOOD OF NORMAL DOGS, WITH SPECIAL REFERENCE TO THE TOTAL VOLUME

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The indirect methods of determining the volume of circulating blood began with Valentine (3) who suggested that the degree of dilution of the solid constituents of the blood might be estimated by injecting distilled water into the circulation, and the blood volume calculated therefrom by means of a formula. The use of many other substances has been subsequently recommended; saline solution, isotonic glucose, Bayliss' solution, foreign serum, and antitoxin. The principle underlying each method is fundamentally the same as that proposed by Valentine.

The value of carbon monoxide as a similar agent was described by Grehant and Quinquaud (3) in 1882. This method was applied to man by Haldane and Smith (4) and subsequently improved by Van Slyke and Salvesen (15) (16).

In 1915 Keith, Rowntree and Geraghty (7) suggested a convenient and reliable method which has found ready acceptance among clinicians and laboratory workers. These investigators introduced vital red into the circulation and after a given period of time withdrew a sample of blood and determined the degree of dilution of the dye in the plasma by colorimetric readings against a known standard. Whipple and his co-workers (6) (2) added refinements to the procedure and showed that many other dyes could be used successfully. Smith (13) devised a modification by which determinations could be repeated at short intervals on the same subject.

In addition to observations on the hemoglobin and red blood cell count, the purpose of the present report is to contribute to the relatively meagre data found in the literature concerning the total volume of circulating blood of normal dogs. The technique of Whipple and his collaborators was followed with the few modifications which are described below.

METHOD. Young, active, male dogs were used throughout. All of the animals were subsequently operated on; the data given below represent routine pre-operative studies.

Counts of the red blood cells were made with standardized pipettes and counting chambers. The percentage of hemoglobin was estimated by the

method of Sahli. Cell counts and determinations of hemoglobin were made from the sample of venous blood withdrawn before injection of the dye. Hematocrit tubes were prepared by using 16.0 mgm. of dried sodium oxalate per 5 cc. of blood. Blood was withdrawn from the saphenous vein and 4 cc. of a 1.5 per cent solution of Congo red were injected through the same needle. The exact time was noted; two and one-half to three minutes later another sample of blood was drawn from the corresponding vein of the opposite limb. (If dye escaped into the subcutaneous tissues the experiment was discontinued.) The samples were centrifuged at 3000 revolutions per minute for one-half hour. The hematocrit values were measured and the plasma from the second sample was read against a standard, prepared with the plasma of the first sample, in a Duboscq colorimeter. The volume of the plasma was calculated by means of the formula and the total blood volume determined by dividing the plasma volume by the value of the plasma hematocrit.

RESULTS AND DISCUSSION. The erythrocyte count and percentage of hemoglobin of adult dogs are uniformly higher than those of man. Similar determinations on the blood of puppies are somewhat lower but approach normal toward the end of the first year of life. Hematocrit values are close to the level found in normal human beings, namely, 45 per cent of cells. This figure is slightly lower than that obtained by Whipple and his collaborators (6) (14), who found a cell hematocrit of 50 per cent or more. This difference may be explained by the fact that our tubes were centrifuged longer and at a higher rate of speed than those of other investigators. Hemolysis occurred rarely. Hemolysed samples were always discarded.

In table 1 are presented individual determinations for each of 25 dogs, average figures for animals in groups including weights of 5 to 10 kgm., 11 to 15 kgm., and 16 to 25 kgm., and the weighted average of all. There were 4 dogs in the first group, 11 in the second, and 10 in the third. The average figures for all animals are practically identical with those of the second group.

In the first group of 4 dogs whose average weight was 8.85 kgm., the red blood cell count was 5.73 millions per cubic millimeter and the hemoglobin was 81.0 per cent. As one might expect from the relatively low erythrocyte count, the cell hematocrit value was slightly lower than the average for all groups, and the plasma hematocrit was correspondingly higher. Consequently the plasma volume and total blood volume were relatively increased and the ratio of blood weight to body weight was 1.8 per cent higher than that of the second group and 1.9 per cent higher than that of the third group.

The average weight of the 11 dogs in the second group was 13.10 kgm., the red blood cell count was 7.85 millions per cubic millimeter, and the

TABLE 1

Tabulation of the individual observations made on each of twenty-five dogs

The ratio of blood weight to body weight was obtained by multiplying the volume of whole blood in cubic centimeters per 100 grams body weight by 1.056, the specific gravity of blood (12). Average figures for groups by weight and for all dogs are true weighted averages.

DOG NUMBER	Hb	R.B.C.	HEMATOCRIT		CELL VOLUME	PLASMA VOLUME	BLOOD VOLUME	PLASMA VOLUME PER 100 GRAM BODY WEIGHT	BLOOD VOLUME PER 100 GRAM BODY WEIGHT	RATIO BLOOD WEIGHT BODY WEIGHT	WEIGHT
			Cells	Plasma							
	per cent	*per cu. mm.*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*				*kgm.*
X^5	98	6,340,000	47.5	52.5	565	625	1,190				
	96	6,460,000	47.9	52.1	530	580	1,110	6.5	12.5	13.2	9.2
X^7	112	7,490,000	50.0	50.0	560	560	1,120				
	111	7,760,000	49.1	50.9	550	570	1,120	4.7	9.3	9.8	12.0
X^{10}	110	7,730,000	41.3	58.7	700	1,000	1,700				
	108	7,500,000	46.2	53.8	810	940	1,750	6.0	10.7	11.4	16.1
X^{12}		8,360,000	51.0	49.0	860	840	1,700				
	118	8,450,000	55.0	45.0	1,020	840	1,860	4.9	10.5	11.1	17.0
X^{13}	80	6,420,000	40.9	59.1	800	1,160	1,960				
	79	6,280,000	38.2	61.8	700	1,135	1,835	8.2	13.5	14.3	14.0
X^{15}	92	6,760,000	44.0	56.0	725	925	1,650				
	96	6,870,000	42.1	57.9	720	990	1,710	5.6	9.9	10.5	17.0
X^{16}	101	6,850,000	46.6	53.4	710	790	1,500				
	102	6,660,000	47.7	52.3	725	790	1,515	4.4	8.3	8.8	18.1
X^{19}	92	7,410,000	45.8	54.2	570	670	1,240				
	94	7,790,000	42.5	57.5	540	730	1,270	5.5	10.0	10.5	12.6
X^{20}	96	6,550,000	49.4	50.6	640	655	1,295				
	92	6,720,000	48.6	51.4	640	680	1,320	5.0	9.7	10.2	13.4
X^{21}	100	7,210,000	46.2	53.8	655	760	1,415				
	96	6,760,000	45.1	54.9	670	815	1,485	6.5	11.9	12.6	12.2
Y^1	80	5,070,000	41.9	58.1	515	735	1,250				
	86	5,450,000	41.4	58.6	520	740	1,260	7.7	13.1	13.8	9.6
Y^5	76		44.0	56.0	420	535	955				
	81	6,380,000	43.8	56.2	405	515	920	7.6	13.6	14.4	6.9
Y^8	111	8,580,000	50.0	50.0	750	750	1,500				
	104	7,530,000	49.5	50.5	745	750	1,495	5.9	11.9	12.6	12.6

TABLE 1—*Concluded*

DOG NUMBER	Hb	R.B.C.	HEMATOCRIT		CELL VOLUME	PLASMA VOLUME	BLOOD VOLUME	PLASMA VOLUME PER 100 GRAM BODY WEIGHT	BLOOD VOLUME PER 100 GRAM BODY WEIGHT	RATIO BLOOD WEIGHT BODY WEIGHT	WEIGHT
			Cells	Plasma							
	per cent	*per cu. mm.*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*				*kgm.*
Y10	88		46.2	53.8	888	1,245	2,133				
	101	6,840,000	45.8	54.2	1,050	1,250	2,300	7.3	13.0	13.7	17.0
Y11	99	7,520,000	47.3	52.7	770	835	1,605				
	96	7,760,000	46.3	53.7	730	850	1,580	6.0	11.3	12.0	14.0
Y13	95	7,770,000	43.9	56.1	1,075	1,380	2,455				
	84	7,200,000	42.1	57.9	940	1,295	2,235	6.7	11.8	12.5	19.8
Y15	60	6,370,000	38.1	61.9	545	885	1,430				
	63	6,030,000	40.0	60.0	530	790	1,320	6.5	10.7	11.3	12.9
Y16	117	8,610,000	47.0	53.0	950	1,070	2,020				
		8,540,000	49.0	51.0	960	1,000	1,960	6.1	11.8	12.5	16.8
Y17	94	6,790,000	42.1	57.9	480	660	1,140				
	90	6,630,000	42.1	57.9	485	665	1,150	5.7	9.8	10.3	11.6
Y18	93	7,330,000	50.6	49.4	1,195	1,165	2,360				
	98	7,000,000	50.1	49.9	1,185	1,175	2,360	6.0	12.1	12.8	19.4
Y19	61	4,630,000	34.5	65.5	400	750	1,150				
	70	5,780,000	34.4	65.6	405	775	1,180	7.8	12.0	12.7	9.7
Y20	80	6,420,000	43.4	56.6	1,115	1,455	2,570				
	80	6,030,000	42.8	57.2	965	1,290	2,255	5.4	9.6	10.1	25.2
H4	93	7,425,000	44.4	55.6	790	970	1,760				
	98	7,450,000	50.0	50.0	860	860	1,720	6.2	11.9	12.6	14.6
H6	108	7,680,000	45.8	54.2	940	1,110	2,050				
	100	7,510,000	48.7	51.3	970	1,025	1,995	6.1	11.6	12.3	17.4
H8	86	6,690,000	44.7	55.3	715	890	1,605				
	94	6,720,000	45.4	54.6	775	925	1,700	6.4	11.6	12.3	14.2
Group 5–10	81.0	5,730,000	41.92	58.08	470	657	1,127	7.40	12.80	13.47	8.85
Group 11–15	92.7	7,085,000	45.26	54.74	659	798	1,457	6.05	11.05	11.67	13.10
Group 16–25	98.4	7,374,000	46.45	53.55	925	1,079	2,004	5.84	10.93	11.56	18.38
All dogs	92.8	7,002,000	45.21	54.79	735	888	1,623	6.18	11.28	11.91	14.53

hemoglobin was 92.7 per cent. The cell hematocrit was 45.26 per cent, a figure which is practically identical with that of man. The plasma volume was 798 cc. and the total blood volume was 1457 cc. The volume of plasma and the volume of whole blood per 100 grams of body weight were 6.05 cc. and 11.05 cc. respectively. The ratio of total blood weight to body weight was 11.67 per cent.

TABLE 2

A comparison of our own results with those of other investigators

The table shows the method used, the number of animals, the range and average weight of each group, the range and average ratio of blood weight to body weight, and the time allowed for mixing, when such information could be obtained.

INVESTIGATOR	METHOD	NUMBER OF CASES	WEIGHT		BLOOD WEIGHT / BODY WEIGHT		MIXING TIME
			Range	Average	Range	Average	
			kgm.	*kgm.*	*per cent*	*per cent*	*minutes*
Brodin, Richet, and Saint Girons (3)	Direct	61	8.0–39.5	20.3	4.7–8.6	6.8	
Plesch (11)	Direct	3	5.8–10.3	8.6	7.8–8.7	8.2	
Grehant and Quinquaud (3)	Carbon monoxide	9	10.1–20.3	18.8	7.7–9.7	8.6*	15
Plesch (11)	Carbon monoxide	6	4.6–14.3	7.9	7.4–10.4	8.8	
Arnold, Carrier, Smith, and Whipple (1)	Carbon monoxide	4	6.9–13.2	10.8	7.2–8.8	8.2*	
Meek and Gasser (10)	Gum-gravity	18	4.6–20.0	8.6	8.3–11.4	9.7	
McQuarrie and Davis (9)	Gum gelatin-refraction	21	7.0–20.5	12.7	7.7–12.0	10.3*	3–5
Hooper, Smith, Belt, and Whipple (6)	Vital red	20	8.5–22.0	15.1	8.7–12.1	10.7*	4
Harris (5)	Congo red	6	7.7–16.5	9.6	7.0–8.2	7.6	
Authors	Congo red	25	6.9–25.2	14.53	8.8–14.4	11.91	2½–3

* These figures have been converted from percentage in terms of volume to percentage in terms of weight of blood. The specific gravity of blood was taken as 1.056 (12).

The average weight of the 10 dogs in the third group was 18.38 kgm. The red blood cell count per cubic millimeter was 7.374 millions and the hemoglobin, 98.4 per cent. The value of the cell hematocrit was 1.2 per cent higher than that of the previous group. Although the plasma volume and total blood volume were considerably increased, the ratio of blood weight to body weight was 11.56 per cent, a figure which is actually lower than that of the preceding group.

The average weight for all groups was 14.53 kgm., the erythrocyte count was 7.002 millions per cubic millimeter and the hemoglobin was 92.8 per cent. The cell hematocrit was 45.21 per cent. The plasma volume and total blood volume were 888 cc. and 1623 cc. respectively. The volume of plasma per 100 grams of body weight was 6.18 cc. and the ratio of total blood weight to body weight was 11.91 per cent.

In table 2, taken largely from Erlanger (3), the results of various investigators are compared with our own. It is apparent that the average weight of our animals is greater than that of other experimenters with three exceptions. Two of these higher averages lie within the "fat dog" range unless the dogs were of exceptionally large breeds; such animals, according to Lee and Whipple (8), have a decreased plasma volume, probably because of fewer capillaries in fat than in muscle.

The time allowed by us for mixing the dye is shorter than that employed by other workers using the same method. On theoretical grounds, the

TABLE 3

A comparison of the results obtained by Whipple and his collaborators using three methods with our determinations by the dye method only

AUTHORITY	AVERAGE WEIGHT OF DOGS	HEMATOCRIT R.B.C.	PER 100 GRAMS BODY WEIGHT								
			R.B.C.			Plasma			Whole blood		
			Dye	CO	Direct	Dye	CO	Direct	Dye	CO	Direct
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*grams*	*grams*	*grams*
Whipple et al (14)	11.55	50.7	5.28	4.39	4.18	5.03	4.21	4.00	10.88	9.08	8.63
Authors	14.53	45.21	5.08			6.18			11.91		

time allotted is sufficient for thorough distribution. Keith et al. (7) found the rate of disappearance of the dye from the blood stream to be 6 to 14 per cent within ten minutes, but felt justified in accepting values determined within six minutes after injection of the dye.

In comparing the relative merits of the three methods, *i.e.*, the use of a dye, the use of carbon monoxide, and the Welcker or direct method, Smith, Arnold, and Whipple (14) came to the conclusion that the first is an accurate measure of the volume of the plasma but not of the cells. The second and third methods determine more accurately the total volume of red blood cells and hemoglobin but not that of the plasma. By adding the volume in cubic centimeters of red blood cells per 100 grams of body weight as determined by the carbon monoxide method to the volume of plasma as determined by the dye method they obtained a figure of 9.2 cc. of blood per 100 grams of body weight, or the "absolute" total blood volume. This includes 0.2 cc. for cellular elements of the blood other

than erythrocytes. By converting this into weight of blood a figure of 9.6 grams of blood per 100 grams of body weight is obtained.

In table 3 their results with the three methods are compared with our determinations by the dye method only.

SUMMARY[1]

1. Observations were made on the blood of 25 young, active, male dogs. The animals ranged in weight from 6.9 to 25.2 kgm.; the average weight was 14.53 kgm.

2. The average erythrocyte count was 7,002,000 cells per cubic millimeter. The percentage of hemoglobin by the method of Sahli was 92.8.

3. Hematocrit values were 45.21 per cent of cells and 54.79 per cent of plasma.

4. Averages of cell volume, plasma volume, and blood volume were 735 cc., 888 cc. and 1623 cc. respectively.

5. The ratio of weight of blood to body weight for all dogs was 11.91 per cent.

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[1] All average figures are true weighted averages.

INCREASED METABOLISM ONLY ONE FACTOR IN THE PRODUCTION AND MAINTENANCE OF THE HYPERGLYCEMIA AND GLYCOSURIA IN EXPERIMENTAL HYPERTHYROIDISM

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Clinicians are inclined to believe that in exophthalmic goitre an excess of thyroid secretion is the exciting cause of the glycosuria; for a lowering of the glucose tolerance with a urinary excretion of sugar may be induced by the medicinal use of thyroid extract.

In view of the complicated physiology underlying carbohydrate metabolism and of the profound effect which the thyroid has upon metabolism in general, no conclusion is justified from data now available as to whether the thyroid gland has any direct influence upon the pancreas as an endocrine organ. Opie (1) is inclined to the view that the glycosuria of exophthalmic goitre is due to an associated lesion of the pancreas and such lesions have been found in some fatal cases. In some cases recorded by Murray (2) the co-existing hyperthyroidism and glycosuria suggests that even when exophthalmic goitre is recovered from, the pancreas may be found irreparably damaged. Opie's interpretation was reported by Cecil (3), who found in ninety cases of exophthalmic goitre alterations in the islets such as sclerosis, hyaline degeneration and leukocytic infiltration.

On the other hand Allen (4) examined the pancreas of 570 human cases with the clinical history unknown to him. He found lesions in 48 per cent of the 549 cases which were clinically non-diabetic. Apparently pancreatic insufficiency does not reveal itself necessarily by morphological or structural changes in the gland.

Fitz (5) and Wilder (6) attribute the lowering or diminution in glycogen to a change in the rate of metabolism and not to the possibility that any hypofunction of the pancreas exists. Wilder is inclined to interpret the hyperglycemia and glycosuria in hyperthyroidism as the effect of increased metabolism.

METHODS. The methods used in feeding, collecting urine and blood, and in quantitatively determining blood and urinary sugar have been described (7) in an earlier article.

Three varieties of partial pancreatectomy were done, namely, removal of $\frac{2}{3}$, $\frac{7}{8}$, and $\frac{19}{20}$ of the gland. In each case the pancreatic remnant was connected to the duodenum through the duct of Santorini.

In trying to bring about the semblance to a hyperthyroid state, the dosage of 0.8 to 1.0 per kilo of desiccated thyroid extract as recommended by Doctor Kunde (8) was tried and found to have the desired effect. The desiccated thyroid was mixed with the animal's food.

TEMPERATURE CHART

TIME	NORMAL	THYROID FED DOG 0.5 GM. PER KILO	15 CC. Na NUC. INJECTED SUBCUTANEOUSLY
	°F.	°F.	°F.
6 a.m.	101.0	101.7	101.6
7 a.m.	101.6	101.6	101.8
8 a.m.	101.6	101.9	103.7
9 a.m.	101.8	101.8	103.8
10 a.m.	101.7	101.9	104.9
11 a.m.	102.1	102.2	106.2
12 m.	102.3	102.6	107.9
1 p.m.	102.2	103.1	107.0
2 p.m.	101.8	102.9	106.3
3 p.m.	101.6	103.0	106.2
4 p.m.	101.3	103.1	104.9
5 p.m.	101.5	103.4	105.3
6 p.m.	101.4	103.6	106.0
7 p.m.	101.4	103.5	104.7
8 p.m.	101.4	104.1	104.6
9 p.m.	101.3	103.6	103.8
10 p.m.	101.7	103.4	104.2
11 p.m.	101.6	103.1	103.9
12 m.	102.1	103.1	104.0
1 a.m.	101.8	102.9	104.8
2 a.m.	101.6	102.8	104.7
3 a.m.	101.7	102.6	104.6
4 a.m.	101.6	102.7	104.0
5 a.m.	101.8	102.4	103.8
6 a.m.	101.7	102.2	103.6

Ten to fifteen cubic centimeters of a 10 per cent solution of sodium nucleinate were injected subcutaneously to induce a high fever (hyperpyrexia).

The degree and duration of the hyperpyrexia induced in normal dogs, thyroid fed dogs, and in dogs injected with sodium nucleinate were determined by taking the rectal temperature every hour over a period of twenty-four hours.

Sections of liver and pancreas were taken during the control, experimental, and the post-experimental periods. Sections of the pancreas were

stained with Bensley's A.O.B. (9), Ehrlich's hematoxylin and formalin Sudan III method for fat for evidences of microscopic changes. Sections of the liver were stained with Best's (10) carmine method for glycogen and the formalin in Sudan III method for fat. In all cases comparison of the microscopic picture was made during the varying stages of the experiment.

RESULTS. *I. The effect of thyroid medication on the blood and urinary sugar of partially pancreatectomized dogs.* A. The effect of feeding large doses of thyroid extract (0.8 to 1.0 gram per kilo to dogs which have been partially pancreatectomized ($\frac{2}{3}$ of the pancreas removed).

BLOOD SUGAR		URINE SUGAR		AVERAGE VOLUME OF URINE	TEMPERATURE	AVERAGE PULSE	RESPIRATIONS
Low	High	Low	High				
Control diet—12 days							
				cc.	*°F.*		
82	90	Neg.	Neg.	257	104.4	84	20
Post-operative control period—4 days							
90	91	Neg.	Neg.	191	101.8	88	22
Fed control diet plus 0.8–1 gram thyroid extract per kilo for 28 days							
84	120	Neg.	Neg.	943	103.2	148	28
Fed control diet without thyroid for 18 days							
82	100	Neg.	Neg.	542	102.6	122	24
Fed control diet with 1 gram thyroid extract per kilo for 39 days							
85	115	Neg.	Neg.	920	103.6	154	30

Except for the change in metabolism which ordinarily accompanies thyroid feeding, there was no evidence of a hyperglycemia and glycosuria. The blood sugar readings fluctuated more widely during the period of thyroid medication, but managed to stay within normal limits. These results confirm the studies of Doctor Kunde (8) on experimental hyperthyroidism.

The only evidence of a microscopic change was a greater fat deposition, perhaps, in the liver, and acinar tissue of the pancreas.

B. The effect on blood and urinary sugar of dogs which were partially pancreatectomized ($\frac{7}{8}$ of the gland removed) and fed large doses of thyroid extract (0.8 to 1.0 gram per kilo).

BLOOD SUGAR		URINE SUGAR		AVERAGE VOLUME OF URINE	TEMPERATURE	AVERAGE PULSE	RESPIRATIONS
Low	High	Low	High				
Control diet—12 days							
				cc.	*°F.*		
88	93	Neg.	Neg.	425	101.4	78	18
Post-operative control period—4 days							
95	112	Neg.	Neg.	344	102.0	82	23
Control diet with 1 gram thyroid extract per kilo for 26 days							
		mgm.	*grams*				
118	178	720	3.25	609	103.6	140	30
Control diet without thyroid extract for 18 days							
88	162	920	2.6	484	102.0	120	26
Control diet with 1 gram thyroid extract per kilo for 39 days							
97	182	720	1.9	719	103.9	152	34

A mild hyperglycemia and glycosuria followed the feeding of large doses of thyroid extract to these dogs. That the hyperglycemia and glycosuria are dependent on the thyroid medication is seen from the fact that when it is removed both the blood and urine sugar are decreased. That a higher hyperglycemia and glycosuria does not follow the second administration of thyroid extract may be due either to an increase in the size of the pancreatic remnant, or a decreased permeability of the kidney to sugar.

Microscopically, the pancreas shows no marked change from the normal. There is an increased amount of fat in the interacinar tissue. The liver shows a decrease in the glycogen as compared to the normal, and a greater increase in fat.

C. The effect of feeding large doses of thyroid extract, 0.8 to 1.0 gram, to dogs in which $\frac{19}{20}$ of the pancreas was removed.

BLOOD SUGAR		URINE SUGAR		AVERAGE VOLUME OF URINE	TEMPERATURE	AVERAGE PULSE	RESPIRATIONS
Low	High	Low	High				
Control period—15 days							
				cc.	*°F.*		
84	96	Neg.	Neg.	250	101.5	86	20

Post-operative control period—5 days							
89	124	Neg.	Neg.	195	102.1	86	18
Control diet with 0.8 to 1 gram thyroid extract per kilo for 21 days							
128	251	840 mgm.	3.8 grams	790	103.1	130	26
Removed thyroid extract for 8 days							
173	200	2.7 grams	3.1 grams	820	103.1	130	26

The feeding of large amounts of thyroid extract produced a marked hyperglycemia and glycosuria, which was hardly diminished with the removal of the thyroid medication. The dogs showed all of the classical symptoms of pancreatic diabetes (polyphagia, polyuria, glycosuria, and polydypsia). They became progressively emaciated and exitus took place in from 14 to 29 days. In the dogs which had been given thyroid extract in the initial part of the experiment (that is, pre-operatively) exitus took place sooner. In the terminal stages of the experiment, the urine gave positive tests for acetone and diacetic acid.

Microscopically, the islets showed no detectable pathologic changes, but the interacinar tissue was filled with fat. The acinar tissue was itself replaced for the most part with fat. Sections of the liver showed a marked depletion of glycogen, and an increased amount of fat.

II. *The effect of subcutaneous injection of sodium nucleinate in dogs which were partially pancreatectomized* ($\frac{19}{20}$).

BLOOD SUGAR		URINE SUGAR		AVERAGE VOLUME OF URINE	TEMPERATURE	AVERAGE PULSE	RESPIRATION
Low	High	Low	High				
Control period—5 days							
				cc.	°*F.*		
84	91	Neg.	Neg.	320	101.4	90	18
Post-operative control period—5 days							
84	115	Neg.	Neg.	208	102.9	94	26
Control diet plus subcutaneous injection of 10 to 15 cc. of Na nucleinate							
118	210	998 mgm.	2.1 grams	380	106.3	124	32

In the experiments of Coleman and Du Bois (11) it was shown that in fever the increase in metabolism and of body temperature occur simultaneously. Sodium nucleinate causes a greater and more prolonged elevation

in temperature than thyroid extract (see temperature chart). It furthermore produces a hyperglycemia and glycosuria in normal animals. Thyroid extract, however, produces a greater hyperglycemia and glycosuria. Animals injected with sodium nucleinate died usually in 14 days.

Here, too, there was no marked microscopic changes from that of the normal. The pancreatic remnant showed no change. In the liver there was a depletion of the glycogen storage. Otherwise the liver showed a normal microscopic appearance.

Discussion. Many patients with Basedow's disease show a heightened blood sugar. In our experiments dealing with experimental hyperthyroidism a hyperglycemia and glycosuria develops only in those dogs which have had the major part of the pancreas removed. Apparently thyroid causes a hyperglycemia and glycosuria only in animals which have a definite pancreatic insufficiency. The following is a résumé of the differences in results obtained from thyroid and sodium nucleinate medication:

1. Sodium nucleinate causes a *greater* and *more prolonged hyperpyrexia than thyroid extract.* (Note accompanying temperature chart.)

2. Sodium nucleinate causes a mild hyperglycemia and glycosuria in normal dogs.

3. Sodium nucleinate causes a hyperglycemia and glycosuria in normal and partially pancreatectomized dogs ($\frac{7}{8}$, $\frac{1}{2}\frac{8}{0}$) only during the periods of its administration.

4. Thyroid extract does *not* produce a hyperglycemia and glycosuria in normal dogs although it causes a fever.

5. Thyroid extract produces a more profound hyperglycemia and glycosuria in partially pancreatectomized dogs ($\frac{7}{8}$, $\frac{1}{2}\frac{8}{0}$) than sodium nucleinate. The hyperglycemia and glycosuria continues as much as seven days after the removal of the thyroid extract. On the basis of these findings it seems permissible to conclude that the hyperglycemia and glycosuria following thyroid administration is not the result of increased metabolism, assuming a fairly direct relation between increase in body temperature and increase in metabolism under the influence of sodium nucleinate. Furthermore, the fever produced in a normal dog fed thyroid is as high as in the partially pancreatectomized. And yet, only a dog with a pancreatic insufficiency develops a hyperglycemia and a glycosuria.

With the factor of safety of the pancreas reduced to a minimum (partial pancreatectomy, $\frac{1}{2}\frac{8}{0}$) heavy thyroid medication induced a condition simulating in all respects experimental pancreatic diabetes. The dogs showed all the classical symptoms of a diabetic state (polyphagia, polyuria, glycosuria, polydypsia). It is our belief that the action of excessive thyroid secretion causes irreparable damage to the pancreatic remnant so as to cause a permanent and complete pancreatic insufficiency. The injection of sodium nucleinate had a fleeting effect. The hyperglycemia and glycosuria

appeared only during the period of injection of the pyrogenic substance. Neither did the sodium nucleinate bring on a permanent glycosuria.

SUMMARY. 1. Large doses of thyroid extract do not produce a hyperglycemia and glycosuria in normal dogs. However, in our series of six dogs which have had the major portion of their pancreas removed ($\frac{19}{20}$) with the factor of safety reduced to a minimum, it was effective in the production of a permanent hyperglycemia and glycosuria (diabetes).

2. The greater hyperpyrexia which accompanies the subcutaneous injection of sodium nucleinate produces a mild hyperglycemia and glycosuria in normal dogs. In our series of four partially pancreatectomized dogs sodium nucleinate did not cause as marked and persistent a hyperglycemia and glycosuria as did thyroid feeding.

CONCLUSIONS

The results seem to show that the influence of the thyroid is not solely through its effect on basal metabolism, but possibly through some toxic effect of thyroxin exerted on the islet tissue of the pancreas. In this sense (toxic action of the thyroid hormone), one might speak of an antagonism between the pancreas and the thyroid.

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THE COMPONENT OF THE DORSAL ROOT MEDIATING VASODILATATION AND THE SHERRINGTON CONTRACTURE

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When a mammalian skeletal muscle is deprived of its proper motor innervation, as for instance by section of the motor roots, and time allowed for degeneration, it acquires a new sensitivity in that stimulation of its nerve, now free of motor neurons, causes a slow muscular contracture which appears after a long latent period and considerably outlasts the stimulus (3, 9, 11, 14, 20, 23). The sensory fibers have been led to exhibit a property which is not at all manifest normally and the question arises as to which of the sensory fibers are responsible. Hinsey and Gasser attempted to solve this problem in the following manner. When a mixed nerve is stimulated with maximal induction shocks and propagation of the response along the course of the nerve is permitted before it is recorded, the nerve action potential is found to be prolonged due to the temporal dispersion of impulses having different velocities. As the slowest fibers have the highest thresholds it was hoped that by progressively increasing the stimulation strengths until the contracture appeared, a simultaneous observation of the potential picture would reveal the fibers involved. The attempt was only partially successful. It was found that long before the contracture appeared the area of the action potential, as it was then known, had reached its maximum value. This could only mean that the action potential in the fibers responsible was somewhere in the wake of the potential wave and of such a magnitude as to cause it to escape detection. The experiment did show however that the dilator fibers must be small fibers as the large fibers had been accounted for in the potential wave recorded. (The basis for this statement is the demonstration by Gasser and Erlanger that it is possible accurately to reconstruct the potential picture from the histological picture for fibers ranging between the upper limit of size and the region of 5μ). Since the active part of the nerve did not reveal itself by its potential sign the only means of identifying it was by its threshold, and therefore accurate measurements of the threshold were made for future reference.

Since the work of Heidenhain on the contracture of the denervated tongue produced by stimulation of the lingual nerve, the association of the contracture with vasodilatation has been recognized. Both require strong shocks and have a long latent period, and the response greatly outlasts the stimulation. The contracture is to be interpreted as being associated with the dilator mechanism rather than with dilatation itself; it is quite independent of any turgescence produced by the latter and represents a real contraction of the muscle fibers themselves. In the case of the limb muscles the dilators involved are those occurring in the dorsal roots; they were first described by Stricker and they have since had their physiology enriched by Bayliss, Ranson and Wightman, Langley and others.

To further test the association with vasodilator fibers, Hinsey and Gasser in some unpublished experiments observed the potential picture in the sciatic nerve of normal cats at the time vasodilatation from stimulation of the dorsal roots became evident. The first sacral root was stimulated while the hind limb was in a plethysmograph and the threshold of dilatation ascertained. Then the plethysmograph was removed and a lead made from the sciatic nerve. Here again as in the Sherrington contracture the action potential area came to its full value before the dilatation appeared and further identification was impossible. The threshold of the response was extremely constant from preparation to preparation and it was measured by recording with the Braun tube oscillograph the shape of the induction shocks employed. At the threshold of the nerve as a whole the potential of the induction shocks was 0.4 of a volt or less, while at the time of appearance of vasodilatation the shock had risen in value to around 10 volts. The dilator fibers are known to be most easily excited by mechanical stimuli and these figures obviously show the inefficiency of induction shocks. The latter are experimentally very useful however because of the degree to which they differentiate fibers of different thresholds.

Since the experiments noted the problem has been given new life by some observations made by Erlanger and Gasser. By the utilization of four panel amplification (about 100,000 times) and a period of observation much longer than the duration of the ordinary action potential, they were able to demonstrate in the dorsal root a slow wave which traveled all the way to the periphery and which in the cat under the ordinary experimental conditions had a velocity of propagation between 1 and 2 meters per second. They designated this wave as the "C" wave to distinguish it from the formerly known wave from which it appeared entirely separate. The fast wave was called the "A" wave. (A "B" is known but it does not occur in dorsal roots. It is of sympathetic origin and as a number of observations have demonstrated that the pseudomotor contracture is not connected with the sympathetic nervous system, it may be at once dismissed as having no bearing on the present problem). The "C" wave as evoked with the same

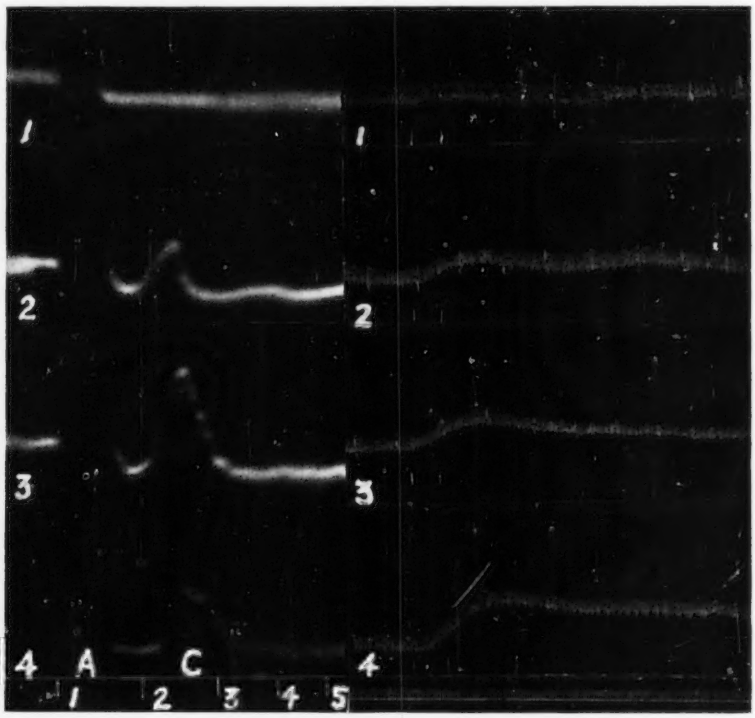
induction coil, which had been used in the vasodilatation experiments, had exactly the same threshold as did vasodilatation; therefore, it seemed very probable that the two would be associated. This led to a reinvestigation of the subject.

VASODILATATION—METHODS. In a normal cat under ether anesthesia the lower lumbar and upper sacral roots were exposed surrounded by their dural sheath. The corresponding hind limb was then inserted in a plethysmograph and connected to a volume recorder of the finger-cot type described by Mendenhall, but constructed with much smaller dimensions. In the earlier experiments the blood pressure was also recorded but as it was never found to change during a procedure this complication was eliminated in the later experiments in order to expedite the electrical recording. When it had been proven that the plethysmograph was functioning, that is, that the base line was sufficiently steady and that the sensitivity was assured by a good pulse tracing, the dura was opened to expose the roots. A dorsal root, usually the first sacral, was cut next to the cord and its proximal end laid on a pair of Harvard shielded electrodes, the latter having been previously arranged so that during break induction shocks the cathode would be turned toward the periphery. The period of stimulation was ten seconds and the shocks were applied at a rate of about twenty per second, the rate being controlled by the rotating contact breaker, which would later be used in synchronism with the recording apparatus for the action potential. After the threshold of dilatation had been determined a series of records was obtained at increasing strengths of stimulation until the response approached maximum. Then the action potentials in the root as elicited by these stimulation strengths were measured. For this purpose the root was cut at its exit from the spinal canal and the cut end killed to render the lead monophasic. Connection was made to the amplifier and the Braun tube oscillograph through Ag-AgCl electrodes, and the rotation rate of the contact-breaker was greatly decreased so that the responses to individual shocks could be observed. The records were in every case of single deflections.

The *results* of these experiments were very uniform and may be very briefly stated. In observations on eight roots in six cats the C wave was found in each case to appear in the potential picture at the strength of stimulation which would first elicit a dilatation. As the vascular response increased the potential also increased, indicating the progressive involvement of more fibers and the distribution of the dilator function throughout all parts of the wave. In this connection it may be mentioned however that a technical difficulty was encountered in many cases, in that the very strongest shocks caused a distortion of the potential picture. The wave was flattened and delayed.

The experiment recorded in figure 1 is a typical sample. The stimulat-

ing circuit consisted of a Harvard-Porter coil with three dry cells in series with six ohms in the primary circuit. The core was in place and a fifteen



I II

Fig. 1. Vasodilatation and the electrical response of the first sacral dorsal root of the cat to stimulation by induction shocks. Reproduction natural size.

Column I. Action potentials. 0.5 m.f., 100,000ω, X = 90, time marked in 0.01 second with the zero at the start of the A wave. Single deflections. Distance of conduction 12.5 mm. A, fusion of the A wave and the shock artefact. C, C wave. 1. Separation of the secondary of the induction coil 9.0 cm. 2, 8.0 cm. 3, 7.0 cm. 4, 5.0 cm., the action potential is delayed at this strength.

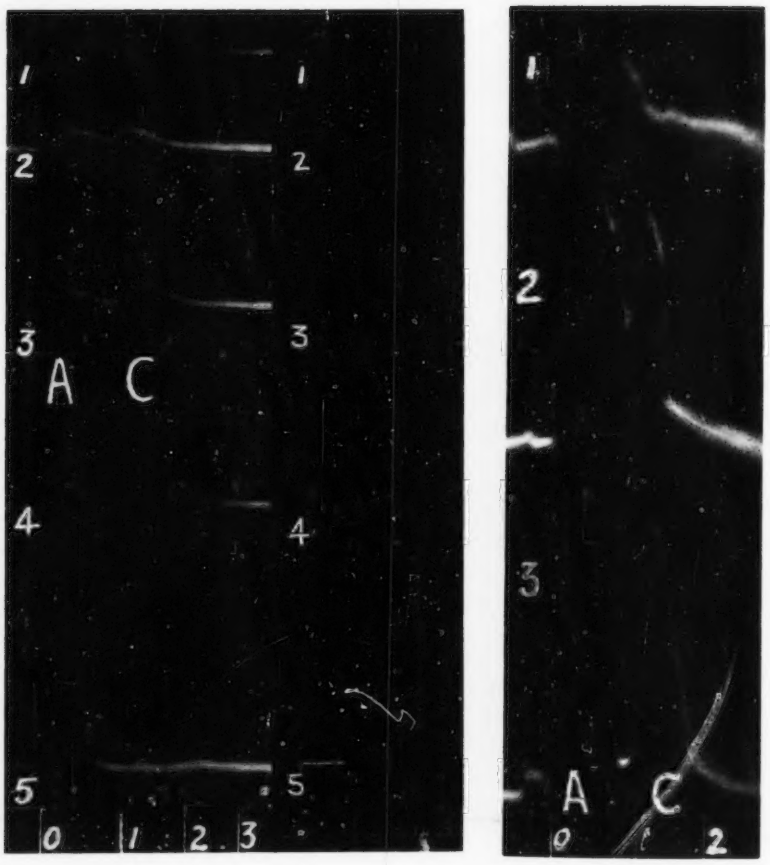
Column II. Plethysmograph tracings from the hind limb. A rise in the line means vasodilatation. Small vertical lines mark the periods of stimulation. The stimulation strengths are the same as in the corresponding figure of column I. Time in seconds.

ohm Helmholz side-wire was employed. Under these conditions both the electrical response and the dilatation were negative when the separation of the secondary coil was 9 cm. At 8 cm. both were well above their thresh-

olds and at 7 cm. they had further increased. At 5 cm. there was a still greater vascular response but the potential picture was obscured by distortion. No attempt was made to limit the threshold further as success in the experiment depended upon expeditious procedure, and further limitation was pointless, as there is nothing else in this region of stimulation strengths with which the fibers in question could be confused.

THE CONTRACTURE. The animals were prepared in advance by cutting the 6th and 7th lumbar and the 1st, 2nd and 3rd sacral dorsal and ventral roots proximal to the ganglia on the right side. Two weeks were allowed for degeneration of the motor fibers and sensitization of the muscle; then the experiments were performed under ether anesthesia. The gastrocnemius muscle was isolated from surrounding structures and suspended, according to the technique which we have previously described, between a a drill, bored through the lower end of the femur, and a tension lever containing a weak spring. The muscle was kept in a sleeve of skin to maintain it in good condition and the experiments were performed in a room whose temperature was 27.5°. The tibial nerve which had been previously exposed and separated from the peroneal was now brought out of the wound, and the proximal end, after being killed, was tied to the distal lead of a pair of Ag-AgCl electrodes. About half way between the end of the nerve and the muscle, shielded electrodes were placed for stimulation. By means of a commutator the cathode during a break shock could be turned either toward the muscle or toward the leading electrodes. The threshold of the contracture was first determined, then a series of contractures was recorded at increasing strengths of stimulation in a manner analogous to that employed in the vasodilatation experiments. Following this the rate of stimulation was slowed so as to permit the recording of single responses and electrical pictures were obtained at the same strengths of stimulation employed in the contracture experiments. To insure the latter the stimulating electrodes were kept in the same positon which they held in the earlier part of the experiment. The only change was that the primary current was commutated so as to have the stimulating cathode on the side of the lead.

The *results* as in the dilatation experiments again displayed the greatest uniformity. The thresholds of the C wave and the contracture were identical and the waves grew side by side as the strength of stimulation was augmented. Successful experiments were performed on five cats. Data from one of them are presented in figure 2. This nerve gave a very fine C wave and it was possible to record it with three panel amplification. The three-panel records are included in the first column. In the third column there are four-panel records corresponding to the upper three in the first column. After each potential record in the first column there is in the second column a contracture record obtained at the same strength of stimula-



I II III

Fig. 2. Responses of the sciatic nerve and the gastrocnemius muscle to induction shocks of various strengths. Cat 13. Roots cut September 28, 1929. Experiment October 11, 1929.

Column I. Action potentials from the tibial nerve, three panel amplification. 0.5 m.f., 130,000ω, X = 100. Time marked in 0.01 second the zero being placed at the start of the A wave. Single deflections. Distance of conduction 14.0 mm. Temp. 27.5°C. A, fusion of A and B waves and the shock artefact. C, C wave. 1. Separation of the secondary of the induction coil, 8.0 cm. 2, 7 cm., 3, 6.0 cm., 4, 5.0 cm., 5, 4.0 cm. The C wave is delayed and prolonged by the strong shocks.

Column II. Contractures produced on stimulation of the tibial nerve, at the strengths of stimulation employed to produce the corresponding potential records in column I. The period of stimulation is marked on the lower record. The periods for the upper records are similar. Time in seconds.

Column III. Action potentials from the tibial nerve recorded with four panel amplification, otherwise as in column I. The artefact shows some variation in form.

tion. Both responses, negative when the secondary coil separation was 8 cm. became positive at 7 cm. The contracture increased up to 5 cm. but the potential change could not be followed quantitatively, on account of a distortion whose origin was not determined. Part of the increase in the contracture at the greatest strength of stimulation may have been due to an increase in rate, due to the possibility that the make shocks had become adequate. In this event the general argument would be in no way affected, however; furthermore when the make shocks are eliminated as in the previous series the same graduation of response occurs.

The closeness of the correspondence of the thresholds of the two types of responses can be seen in figure 3. By watching the screen of the

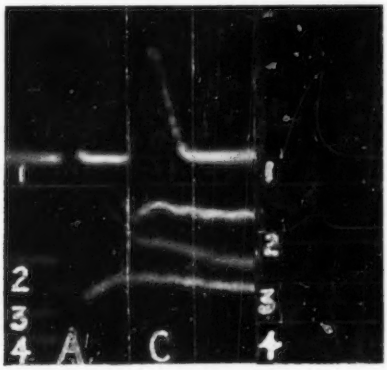


Fig. 3. Showing the exactness of the correspondence of the thresholds of the C wave and the contracture, when the tibial nerve is stimulated. I. Action potentials, single deflections, four panel amplifications. II. Contracture. A and C as in figure 2. Secondary coil distances; 1, 7.0 cm. 2, 8.8 cm. 3, 9.0 cm. 4, 9.2 cm. The contracture at 7.0 cm. is still submaximal. The artefact is variable.

Braun tube it was possible accurately to predict the contracture produced by that particular strength of stimulation when applied at the proper rate. With the coil at 9.2 cm. there were no responses (line 4). When the shock was strengthened to 9.0 cm. it can be seen that there was a trace of an effect (line 3) in both records, and when it was further increased to 8.8 cm. the responses became more definite (line 2). At 7.0 cm. both responses were well developed (line 1) but they were still far from maximal.

Discussion. These experiments demonstrate that vasodilatation of dorsal root origin and the pseudomotor contracture of sensitised mammalian muscle are mediated by fibers having the whole range of thresholds obtaining for the group which is responsible for the C wave of the action potential. This fact permits a number of conclusions to be drawn.

In the first place dorsal root dilatation is shown to be confined to a very restricted group of fibers, a group of fibers conducting at a velocity around two meters per second. No attempt has been made in this series to determine exactly their *in vivo* velocity. Erlanger and Gasser found velocities between 1 and 2 meters per second for the same fibers when the nerve was in the incubator at 37°. In this series in some nerves velocities faster than that were seen even when the nerve was exposed to the room temperature of 27.5°. The greater velocity must have been due to a better condition of the nerve; it was found when the C wave was high and short as in figure 2, column 3, records 2 and 3. When the nerve deteriorated the wave became much longer and flatter and the maximum velocity fell to half a meter per second. Since the dilatation is restricted to the C wave, it is obvious that it cannot be produced as an axon reflex from any of the sensory fibers contained in the A group, either from the group which supplies the proprioceptive fibers to muscle or from the group which goes to the skin.

In the second place information is derived as to the constitution of the C wave. Erlanger and Gasser came to the conclusion that the C fibers are fundamentally different from A fibers in composition. The size of the C fibers cannot be predicted from their velocity of conduction in comparison with A fibers as an histological analysis of a root does not yield the necessary information. It is not even known whether they are myelinated or unmyelinated; the only evidence is that they are small and this is the negative evidence that the large fibers are accounted for in the A wave. It is at present only possible to identify a C fiber when one of its functions has been found, therefore Hinsey's observation that some of the dorsal root innervation of the arterioles of muscle is by medullated fibers of about 4 to 5μ establishes the fact that some at least of the sensory C fibers in the cat may be of the medullated variety.

Finally the fact that both dilatation and the contracture are associated with the C wave strongly supports the supposition that they are interrelated. This point needs further discussion. If the contracture be not dependent on the dilator fibers, some other fibers must be found. One possibility is that the C fibers might have a direct connection with the muscle fibers, but as we pointed out in our previous paper, the behavior of the muscles is not like that which occurs on indirect stimulation, it resembles rather that evoked by pharmacodynamic means. Furthermore Hinsey (1927) has found that no small nerve fibers of dorsal root origin end hypolemmally in muscle fibers.

Without a C fiber connection with the muscle fibers, the only possible way the muscle could be affected would be by some humoral substance formed outside of it. In our previous paper, we proposed that if vasodilatation be humoral in nature that the vasodilator substance might be effec-

tive. After the paper was in press, we discovered that Bremer and Rylant had previously proposed the same theory and that Langley in a vaguer way had held the same view before that. Since then the idea has occurred independently to Dale, so it is evident that every one who considers the subject is forced to the same conclusion.

In order to support such a theory we must first make sure that the dorsal root vasodilator fibers go to muscle. A brief survey of the literature brings to light ample evidence that such is the case. In the first place the dilatation has been observed. Bayliss obtained dilatation in the leg of the dog after the skin was removed, though it was not as great as in an intact limb. Also Krogh, Harrop and Rehburg observed capillary dilatation, and in one case arteriolar dilatation when the dorsal roots were stimulated in the frog, though again the dilatation was not as great as the corresponding effect in the skin. Secondly, Hinsey (1928) has described dorsal root fibers which pass into muscle to end in the connective tissue between the muscle fibers and in the adventitia of blood vessels. In his figures 9 and 10 is shown a small myelinated fiber which gives rise to an unmyelinated branch which passes down to terminate in the adventitia of a terminal arteriole. The close proximity here of the nerve end, the arteriole and the muscle fibers makes it apparent that if some chemical substance be formed either in the nerve fiber or in the arteriole as the result of the action of the fiber, that substance would have ready access to the muscle fibers. Finally Erlanger and Gasser have demonstrated the C wave in the muscle branches of the femoral nerve of the dog.

All the C fibers going to muscle are in all probability not dilator fibers however. Erlanger and Gasser in their discussion of the C wave in the dorsal root point out its possible association with the bundle of small fibers which goes to make up the zone of Lissauer and they thereby admit that it could carry the functions attributable to the Lissauer zone as the result of Ranson and Billingsley's experiments, notably pain and nociceptive reflexes. Pain sensations might therefore travel to the central nervous system in the C waves; but it is difficult to see how this fact could have any bearing on the production of the contracture except as the dilator fibers might also be pain fibers and the pain branch might be the afferent arc of an axon reflex.[1] Such a relation to pain does not seem very probable and it is quite possible that the branches of the dilator fibers necessitated by the axon-reflex theory may be excited in some other way. There are small

[1]In accord with the prevailing view the vasodilators are considered to be normally brought into activity by an axon-reflex. But it is not intended thereby summarily to deny the possibility of vasodilator reflexes in the face of the positive experiments of Bayliss and of Fofanow and Tschalussow. These experiments have not been successfully explained away; the disfavor which they encounter is due to the fact that fibers with their trophic centers in the dorsal root ganglia would have to be accessible to stimulation within the spinal cord.

fibers which terminate in endomysial connective tissue which might serve that purpose; however they might also themselves directly or indirectly serve as the source of a substance.

All the possibilities necessitate the postulation of humoral intervention. We cannot here cite all the evidence supporting the humoral transmission theory but one experiment will be mentioned which gives it the strongest support in the case of antidromic dilatation. Lewis and Marvin have found that, if the stimulation take place during a period of ischemia, the flush lasts as long after the return of the blood as it would have lasted with the blood flow intact. Since this is not true for dilatation in general they conclude that the substance in this case is derived from the tissues and they believe it to be histamine-like. These conclusions which have a logical enough basis do not however preclude the possiblity of the formation of chemical substances in a more reversible manner in the blood vessels nor the existence of other substances than histamine. The substance histamine itself can have no significance in our problem as it is quite without a contracture producing action in sensitized mammalian muscle (Dale and Gasser).

Doi has shown that antidromic dilatation in the frog involves both capillaries and arterioles. When the capillaries were maximally dilated by histamine, he found that dorsal root stimulation caused dilatation of the arterioles and conversely, when the arterioles were maximally dilated with acetylcholine, stimulation caused dilatation of the capillaries. Interpreted on a humoral basis, these important experiments necessitate the assumption of more than one substance. The histamine-like substance of Lewis and Marvin would explain the capillary dilatation in the leg treated with acetylcholine. But in the case of the leg treated with histamine clearly another agent must be sought and it is natural to suppose, in order to make the argument symmetrical, that the agent is acetylcholine. Until recently the latter substance could not be brought into the reckoning as it was not known to exist in the body, but the situation has now been changed on account of a brilliant contribution to the subject by Dale and Dudley. The latter have isolated for tissues the acetyl ester of choline in pure form and Dale now proposes that the humoral link is actually acetylcholine itself.

In every detail but one this proposal is beautifully in accord with the facts. Acetylcholine liberated from the region of the blood vessels would act on the skeletal muscle cells like the same substance added from without. A contracture would be produced by its nicotine-like action (3) and there would be no antagonism by atropine. The difficulty arises in connection with acetylcholine as the humoral substance responsible for dilatation. This is due to its muscarine-like action and is vigorously antagonized by atropine, whereas antidromic dilatation, as shown by R. Hunt, is not. To surmount this difficulty, Dale has made use of the subsidiary hypothesis

that acetylcholine as it is physiologically formed is not accessible to atropine.

SUMMARY

Antidromic dilatation produced in the cat by stimulation of the dorsal roots is mediated entirely by a group of fibers responsible for the C wave of the action potential picture. These fibers have a velocity of conduction in the region of two meters per second.

When a muscle has been sensitized by destruction of its motor innervation, the contracture produced by stimulation of its sensory innervation (the Sherrington contracture) is also mediated by fibers responsible for the C wave.

These facts taken together are strong evidence that the fibers involved in the two cases are identical and that the contracture is dependent upon the dilator mechanism. A discussion of this point is given.

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ACTION OF PHLORHIZIN ON HUNGER CONTRACTIONS IN THE NORMAL OR VAGOTOMIZED DOG

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It has been noted by Luckhardt (quoted by Carlson, 1916, 230) that many of the conditions which increase hunger contractions have two things in common: 1, acidosis of varying degrees, and 2, either inability to use sugar by the tissues or else a lessened amount of sugar available for the use of the tissues because of the sugar having been oxidized or eliminated. Experiments by Luckhardt indicated that phlorhizin diabetes produced augmentation of gastric motility and probably acted by the above mechanisms. In an attempt to determine the rôle of the blood sugar level in the control of gastric hunger activity we have considered it desirable to extend the experiments of Luckhardt. Since Quigley and Templeton (1929) have shown that insulin hypoglycemia produces an effect on the motility of the vagotomized stomach which is the opposite of that obtained on the stomach of the normal dog it seemed desirable to compare the action of phlorhizin on the vagotomized stomach with its action on the normal stomach.

From experiments performed on the vivi-perfused stomach of phlorhizinised dogs Lim, Ni, Necheles and Chang (1929) were unable to evaluate the degree of motility of their stomach preparations with any exactness from the tracings recorded, but in their experiments there was no obvious difference in the degree of motility exhibited by the phlorhizinised and normal stomach during the control period.

METHODS. The experiments were performed on normal dogs and on dogs whose vagi were sectioned just above the diaphragm. These animals were trained to lie on a table while records of gastric motility were obtained by means of a rubber balloon introduced into the stomach by way of the esophagus or through a gastrostomy opening. In three of the series of experiments motility was recorded from three parts of the stomach by means of the triple balloon method. With each dog a tracing was made each day with the animal fasting for twenty hours at the beginning of the record and was continued for a period of 3 to 4 hours. After completion of the record the animal was fed a standard meal (slightly less than the

maintenance requirement) and it was noted in each case that the food was all eaten within 10 minutes of the time of feeding. On a few occasions the animals were each given a liter of water by stomach tube about 16 hours after being fed. This was done to aid in removing from the stomach food residues which might exert a depressant action on the hunger contractions. There was no indication that this procedure modified the results or that the stomach on any occasion contained food during the period of recording the gastric motility.

Gastric activity was recorded daily for a control period of 6 to 10 days, then the phlorhizin was administered and the records continued for 15 to 25 days more. On six normal and two double vagotomized dogs we studied the effect of one injection of one gram of phlorhizin suspended in olive oil. In another series of experiments the same animals were used to determine the effect of the administration of one gram of phlorhizin in oil and one gram in sodium carbonate solution injected each day for five days (total of 10 grams), a procedure which the work of Deuel, Wilson and Milhorat (1927) indicates would produce a maximal phlorhizin effect. The phlorhizin used in this investigation was Merck's preparation purified by the method suggested by Lusk (1928 p. 626). In some of the experiments the "true" blood sugar was followed by the method of West, Scharles and Peterson (1929)[1] and the urine was also analyzed for sugar. The material for the analysis was collected each day at the end of the period during which the gastric motility was recorded and when phlorhizin was administered these injections were made at approximately the same time.

Results. The administration of phlorhizin modified gastric motility in a similar manner when given in 1 gram or 10 gram amounts and when given to normal or vagotomized animals. Inhibition of motility, rather abrupt in onset was usually the first change in gastric activity observed. The duration of this inhibition varied somewhat but in general agreed well with the period of glycosuria and hypoglycemia. Following the administration of one gram of phlorhizin the inhibition lasted for about 6 days (4 to 10 days) and when the ten grams were given the inhibition lasted for about 10 days (9 to 15 days). During the period of gastric inhibition the animals in addition to the glycosuria and hypoglycemia, displayed some anorexia, became more sluggish and lost somewhat in weight. In some of the animals, ulcers developed at the site of the oil injections but since these made their appearance only during the later part of the inhibition period it is believed that they had little if any effect on the gastric motility. Furthermore, the gastric motility from these animals displayed the same variations as those noted in animals in which ulcers did not occur. Following the period of gastric depression there was usually a period of gastric

[1] We are indebted to Mr. E. I. Solomon for the blood sugar determinations

stimulation during which the activity was greater than during the control period. At this time the glycosuria had disappeared, the blood sugar had returned to normal, the animal was much more lively and displayed a vigorous appetite. The experiments were only continued for six days following the period of gastric depression and were not prolonged sufficiently to determine exactly how long the augmentation in gastric activity would persist.

The gastric activity displayed by the animal during the control period was considered from the standpoint of frequency and height of contractions, persistence of activity, frequency of periods of activity and gastric tone and the average type of activity occurring during this interval was arbitrarily called ++++ (4). Activity occurring during the remainder of the experiment was compared with the control activity and classified

TABLE 1

Average type of gastric activity arranged in periods

ONE GRAM OF PHLORHIZIN				TEN GRAMS OF PHLORHIZIN			
Number of experiment	(6–10) Control	Phlorhizin depression	(6) Recovery	Number of experiment	(6–10) Control	Phlorhizin depression	(6) Recovery
1	4	(9) 3.0	4.3	7	4	(15) 2.7	4.7
2	4	(5) 3.2	4.0	8	4	(13) 2.8	4.7
3	4	(6) 3.5	5.5	9	4	(10) 3.0	5.4
4	4	(4) 3.4	5.2	10	4	(10) 2.7	5.4
5	4	(7) 3.9	4.1	11	4	(10) 2.4	4.8
6	4	(7) 4.0	5.0	12	4	(15) 4.0	5.4
13	4	(6) 3.8	4.3	15	4	(11) 2.1	4.7
14	4	(7) 3.6	4.3	16	4	(9) 3.2	4.4

Numbers in ( ) signify days of period.

Experiments 1 to 12 normal dogs, 13 to 16 double vagotomized dogs.

according to whether it was of greater or lower intensity. Throughout the entire series of experiments no changes in types of activity other than those noted above were observed. The most important changes were a decrease in activity and (to a lower degree) of tone during the period immediately following the phlorhizin administration and an increase in activity and tone during the recovery period. The records obtained with the triple balloon indicated that all parts of the stomach responded to the experimental procedures in a similar manner.

Discussion. The results definitely show that under the experimental conditions a decrease in motility of the fasting stomach occurred during a time when the blood sugar and the glycogen reserve were reduced and a condition of acidosis was present and furthermore, as these conditions disappeared gastric motility was augmented. In other words, when phlorhizin was present in relatively high concentrations in the body the dominant

action on gastric motility was depressant. Several explanations of this result may be considered: (1) Quigley and Templeton have noted that the hypoglycemia produced by insulin gives rise to inhibition of the vagotom-

TABLE 2

Protocols of two experiments (normal dogs fasting for 20 hours)

	EXPERIMENT 1 (1 GRAM PHLORHIZIN)					EXPERIMENT 8 (10 GRAMS PHLORHIZIN)			
	Date	True blood sugar	Urine sugar	Gastric motility	Average motility	True blood sugar	Urine sugar	Gastric motility	Average motility
Control period	6–11	79.0	0	+++		77.5	0	++++	
	6–12	73.2	0	++++		72.0	0	+++++	
	6–13	86.4	0	++++		82.4	0	++++	
	6–14	75.0	0	++++		73.2	0	++	
	6–15	77.9	0	+++++		79.0	0	++++	
	6–16	80.3	0	++++*	4	75.0	0	+++++**	4
Period of phlorhizin Gastric depression	6–17	74.0	++++	+++		74.4	++++	++**	
	6–18	65.0	++++	+‡		62.0	++++	+**‡	
	6–19	57.6	++++	+++‡		55.0	++++	++**‡	
	6–20	50.0	++++	+++		48.2	++++	+++**‡	
	6–21	52.0	+++	+++		48.2	++++	++‡	
	6–22	46.0	++	++++		43.2	++++	+++	
	6–23	53.8	++	+++§		40.6	++++	++	
	6–24	45.6	0	++++		52.8	+++	+++	
	6–25	58.2	0	+++	3.0	56.0	+++	+++	
Period of recovery	6–26	78.8	0	++++¶		52.8	+++	++++	
	6–27	85.0	0	+++++¶		46.8	+++	+++	
	6–28	86.0	0	+++		68.4	++	++++	
	6–29	84.3	0	+++++		62.0	+	++++	2.8
	6–30	79.8	0	+++++		75.6	0	+++++	
	7–1	75.0	0	++++	4.3	80.1	0	++++	
	7–2					86.0	0	++++¶	
	7–3					78.0	0	+++++¶	
	7–4					79.2	0	++++++	
	7–5					82.4	0	++++	4.7

* Injected 1 gram phlorhizin in oil.

** Injected 1 gram phlorhizin in oil, 1 gram in Na_2CO_3.

‡ Mild depression, moderate anorexia.

§ Small ulcer at site of injection.

¶ Condition very good, lively.

ized stomach. It may be that in the normal animal phlorhizin produced vagus depression, then in both the normal and the vagotomized animal the hypoglycemia may lead to gastric inhibition through splanchnic stimulation; 2, lowered glycogen reserve and blood sugar and a condition

of acidosis may not be the important factors in augmenting hunger contractions; 3, the administration of phlorhizin may have given rise to an inhibitory effect which was able to overcome any augmentatory action of other factors. The period of augmented gastric motility is probably to be explained as the natural response which the stomach would make to any procedure which would reduce the animal's food reserves but this augmentation could not become manifest while phlorhizin was present in the body in large amounts. Since the results in vagotomized animals are similar to those of normal dogs it suggests that the depression and also the augmentation which follows phlorhizin administration occurs through a direct action on the peripheral motor mechanism. In the completely denervated (vivi-perfused) stomach preparation Lim, Ni, Necheles and Chang did not observe changes similar to those described above. This would tend to contradict the possibility of peripheral action. The normal variations in the motility of various vivi-perfused stomachs, however, are probably as great as those produced in our experiments by phlorhizin.

We take pleasure in expressing our gratitude to Prof. A. J. Carlson for helpful criticism and for facilities freely offered.

SUMMARY

Administration of phlorhizin to normal or vagotomized dogs produces inhibition of gastric hunger contractions during the period of glycosuria and hypoglycemia and when these conditions disappear the gastric motility returns to normal or goes above it.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

VI. The Effect of Crotalin on a Visceral Organism

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Accompanying the marked fall in blood pressure which characterizes the action of crotalin there is invariably a decrease in the volume of the splanchnic viscera and an increase in the volume of the hind limb. After a varying length of time the splanchnic viscera show a marked increase in volume, and at necropsy these organs are intensely congested and frequently are observed to be edematous.

In order to decide whether the initial decrease in volume of the splanchnic viscera was secondary to the low blood pressure, or whether the diminished blood flow to the abdominal organs was secondary to an extensive shunting of blood to the periphery, a modification of Carrel's visceral organism was used (Markowitz and Essex, 1930).

A visceral organism is a preparation consisting of the thoracic and abdominal viscera removed from the body and kept alive by artificial respiration. It is prepared by tying and sectioning systematically all the blood vessels that connect the aorta and the vena cava to the parietes. The appearance of these organs is relatively normal and for the purpose of this research a visceral organism may be considered as an animal devoid of skeletal muscle and central nervous system. It follows that if the shrinkage of the splanchnic viscera after an injection of crotalin is an active phenomenon, the visceral organism should react to crotalin by an initial rise in blood pressure.

Experimental methods. The visceral organism was prepared by the usual technic. A cannula was inserted into the brachiocephalic artery and connected to a mercury manometer in the usual manner except that heparin in Ringer-Locke's solution was employed as the anticoagulant in the connecting tubing. Intravenous injection of 0.2 cc. of 4 per cent crotalin was followed by a marked rise in blood pressure from 70 mm. to more than 100 mm. and was followed after several minutes by a gradual fall to about 40 mm., the level observed in a crotalin-shocked animal.

Accompanying the pressor action of crotalin was a marked increase in the activity of the intestine. The stomach regurgitated its contents through the esophageal tube. The ileum violently expelled its contents through the drainage tube. The heart rate, when the blood pressure had almost risen to its peak, became accelerated. These effects slowly disappeared and the amplitude of the cardiac pulsations on the record of blood pressure slowly decreased as the blood pressure fell. The intestines became very congested and cyanosed. The spleen became enlarged. The experiment was repeated three times, with identical results.

COMMENT. Since the visceral organism is a preparation that has been removed from the body and placed in an incubator, there can be no question that the results obtained are not central. They indicate that when crotalin is injected into an intact dog the resultant defecation and vomiting are not necessarily central in origin.

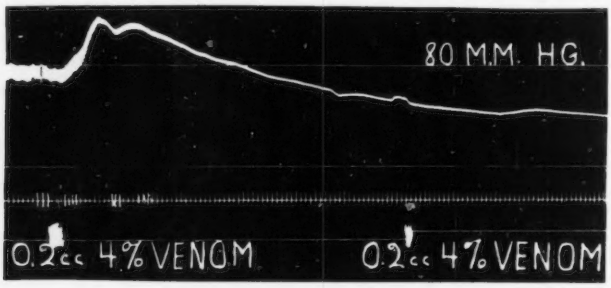

Fig. 1. Effect of 0.2 cc. of 4 per cent venom on the blood pressure of a visceral organism. Time marker records every four seconds. Above the time marker is the zero base line.

The experiment also proves that the fall in blood pressure in an intact dog is not cardiac in origin, since if it were, crotalin would act similarly on the visceral organism. The fact that the blood pressure of a visceral organism is raised by crotalin may be considered as evidence that the decrease in the volume of the splanchnic viscera, which takes place in an intact animal after the injection of crotalin, is the result of active contraction of the splanchnic arterioles, and is not the result of low blood pressure per se or shunting of blood to the periphery. The behavior of the blood pressure of the visceral organism exactly corresponds with the plethysmographic observations of these structures in the relatively intact dog. During the interval which in an intact dog corresponds to the shrinkage of the splanchnic viscera, the visceral organism shows a rise in blood pressure. During the period in which the blood pressure of a visceral organism falls markedly, the splanchnic viscera of the intact dog show marked increase in volume.

As to the mechanism of the pressor action of crotalin on the visceral organism we have little to say. The fact that the action is almost immediate and is accompanied by hyperactivity of the intestine indicates that it is not the result of secondary liberation of epinephrine.

Whatever the ultimate explanation offered for the fall in volume of the splanchnic viscera from injecting crotalin into an intact dog, our studies on the visceral organism prove that these changes reside in the viscera.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

VII. The Similarity of Crotalin Shock and Anaphylactic Shock

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The physiologic alterations produced in an organism by the intravenous injection of crotalin bear much resemblance to histamine shock and more especially to anaphylactic shock. It was only after a prolonged series of diverse experiments that we obtained sufficient evidence to conclude that crotalin shock and anaphylactic shock were not identical in their physiologic manifestations. In the dog, the clinical symptoms of crotalin shock are indistinguishable from those seen in anaphylactic shock. For a time we explained the postmortem data in severe crotalin shock on the basis that the reaction was merely an extreme example of the same vascular reaction exhibited in anaphylactic shock, and the more fully we considered this condition, the more likely it seemed that the mechanism of shock in a sensitized animal, following the requisite dose of antigen, was identical with the mechanism of shock following an injection of crotalin. In view of the fact that anaphylaxis is held by some to be the result of the development of anaphylotoxin, we consider it advisable to place on record the results of our investigation, since it will be evident that a proponent of the anaphylotoxin theory could have made a good case for the classification of crotalin as an anaphylotoxin. This substance has practically all the properties that one would expect of theoretic anaphylotoxin.

The following are the points of similarity of crotalin shock and anaphylactic shock: The administration of antigen to a suitably sensitized dog is followed by a sudden profound fall in blood pressure, accompanied by vasconstriction of the spleen, kidney and intestine (Biedl and Kraus, 1909, and Pearce and Eisenbrey, 1910). This fall in blood pressure is peripheral, and definitely neither central nor cardiac. At necropsy the liver is congested and there are hemorrhagic areas under the serosa of the peritoneal organs. Similarly, the intravenous administration of crotalin to a dog is followed by a fall in blood pressure which is neither cardiac nor central in origin, and which is accompanied by shrinkage of the liver, spleen, kidney and intestine. At necropsy, these organs are congested, and in animals

that have died quickly after the injection, the observations at necropsy may be identical with those observed in anaphylactic shock. The hemorrhages in the submucosa of the intestine are alike in both conditions, and several hours after shock the blood is usually incoagulable.

The addition of venom to the fluid used for perfusing the excised uterus of the virgin guinea pig regularly evokes maximal contraction of this organ; the addition of antigen, under the same circumstances, provokes contraction of the uterus of the sensitized guinea pig. The differences in the reaction, which have been reported previously (Essex and Markowitz, II), are not fundamental.

When crotalin is injected intravenously into a guinea pig, the animal shows signs of labored breathing, and wheezes and râles become audible to the unaided ear. Occasionally, the animals scratch at their noses. At necropsy, the lungs are distended and the histologic appearance is that of acute emphysema, with rupture of the interalveolar septa and edema and corrugation of the bronchiolar mucous membrane.

In view of these observations, it became desirable to obtain more precise information regarding the reaction of the lung of the guinea pig to crotalin, and also to obtain information on the following phenomena: 1, the formation of precipitate in the serum of normal dogs on incubation with crotalin; 2, the marked rise in pressure within the bladder following intravenous injection of crotalin in the etherized dog,[1] and 3, the nature of the reaction following the intradermal inoculation of dilute crotalin into the skin of human subjects.

Since the fixation of the lung of the guinea pig is the most usually accepted criterion of anaphylactic shock, we are reporting the effect of crotalin on the pulmonary volume of guinea pigs. The technic of Jackson, as adapted by Koessler and Lewis (1927), was employed with slight modifications. The method will allow detection of degrees of sensitization that are far too slight to be detected by the clinical observation of a guinea pig. The method, as we have used it, is as follows:

Sufficient procaine is injected under the skin of the occiput, the skin is incised, a probe is quickly passed through the occiput, and the pithing of the brain is completed. The spinal cord need not be pithed. A tracheal cannula is then rapidly inserted and intratracheal insufflation of oxygen or air is begun. The oxygen is offered to the lungs at a constant positive pressure of 10 mm. of mercury, and the stream is interrupted about thirty times a minute. As the insufflation is interrupted, the lungs become deflated by virtue of their own elasticity. This expansion and deflation of the lungs is recorded through a heavy needle which brings both pleural cavities into communication with a delicate tambour that writes on a smoked

[1] Manwaring, Marino and Boone (1927) pointed out that this is a characteristic phenomenon in the anaphylactic shock of dogs.

drum. The needle, with its stilet in position, is introduced in the sixth intercostal space on the right side and is pushed ventral to the heart into the left pleural cavity. The shaft of the needle contains several apertures, so that the changes of pressure of both lungs are recorded as one. As the

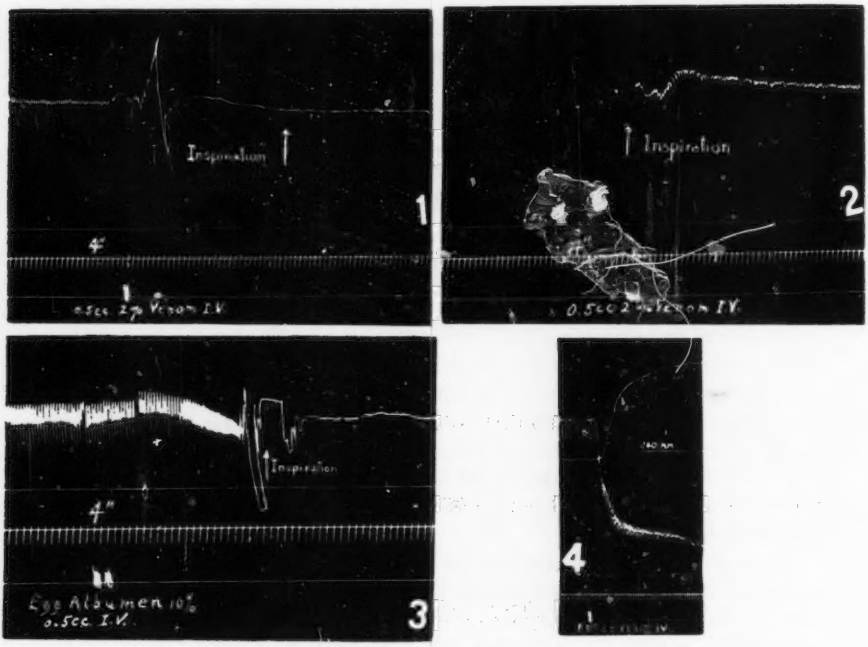

Fig. 1. Pulmonary volume of pithed guinea pig showing complete bronchial spasm resulting from the intravenous injection of 0.5 cc. of 2 per cent venom. At necropsy the lungs were found to be markedly distended.

Fig. 2. Pulmonary volume of pithed guinea pig. The bronchial spasm was incomplete following intravenous injection of 0.5 cc. of 2 per cent venom.

Fig. 3. The pulmonary volume of the guinea pig sensitized to egg albumin, showing the typical complete bronchial spasm following the intravenous injection of 0.5 cc. of 10 per cent egg albumin.

Fig. 4. Intravesical pressure of a dog following intravenous injection of 0.017 cc. of undiluted venom. Upper tracing, blood pressure; lower tracing, intravesical pressure.

lungs are inflated, the writing point moves up. As the lungs deflate, the reverse occurs. In the event of bronchial constriction, the amount of air entering the lungs is curtailed. When there is occlusive bronchospasm, this is indicated by complete cessation of movement of the writing point. At the end of each experiment in which occlusive bronchospasm did not

occur, the apparatus was tested by injecting 0.1 mgm. of histamine intravenously. Complete bronchospasm is shown in figure 1, and incomplete bronchospasm is shown in figure 2. The intravenous administration of the white of egg to a sensitized guinea pig is promptly followed by occlusive bronchospasm, which cannot be overcome by increasing the air pressure up to 30 and 40 mm. of mercury (fig. 3). The same is true following the injection of histamine, although in an occasional case, the histamine brings about bronchiolar constriction which is not occlusive and which is manifested by diminution in the excursion of the writing point to about one-third. The injection of crotalin intravenously in more than twenty cases resulted in occlusive bronchospasm in about a third of the cases; in about a third of the cases there was considerable curtailment of the inflow of air, and in the remaining cases there was some bronchial constriction, as evidenced by temporary diminution in the excursion of the writing point.

It is clear from the tracings that the reaction of the bronchioles of the guinea pig to crotalin is in accordance with the similar reaction of this animal to histamine and to anaphylaxis. The fact that occlusive bronchospasm can be provoked in a considerable number of guinea pigs by crotalin, distinctly puts it into relation with anaphylactic shock. At first sight, this observation lends support to the anaphylotoxin theory of anaphylactic shock. It will be recalled that there are two theories for the mechanism by which an antigen is violently poisonous when injected into a sensitized animal. According to the first one, propounded by Besredka, and ably developed by Dale and Kellaway (1922) the anaphylactic condition is due to the union of antibody and antigen in the protoplasm of the cells of the body. Dale has brought forward substantial evidence for this view.

In brief, the Dale-Besredka hypothesis of anaphylactic shock is that antigen unites with precipitin in the cells of the body, and that the resultant colloidal upset is the basis of shock. If the precipitin titer of the blood is sufficiently high for the blood to unite with the antigen before it gets to the cell, shock does not occur; similarly, if there are no precipitins in the animal for the antigen, there is no shock, which is the case in an animal that has not previously been sensitized. According to the anaphylotoxin theory, if an antigen is injected into an animal that is sensitized properly, a poisonous substance develops which gives rise to the symptoms of shock. This poisonous substance is elaborated as a cleavage produced from the antigen by a specific ferment the appearance of which in the blood stream is provoked by the sensitizing injection. In favor of this hypothesis is the large number of substances that can be manufactured from blood serum and that can be shown to produce apparently characteristic death when a few cubic centimeters are injected into a guinea pig.

It must be apparent, at this stage of our description of the physiologic properties of crotalin, that the substance could readily be classified as an

anaphylotoxin by a proponent of that theory. We wish to point out, however, that the degree of endothelial poisoning which results from the intravenous injection of crotalin is much greater than one sees in anaphylactic shock. Moreover, the symptoms resulting from the intravenous injection of crotalin in the guinea pig are not usually predominantly asphyxial, although there is some evidence of respiratory distress in nearly every case.

We do not wish to state that crotalin shock is the same as anaphylactic shock since the latter condition is by definition the violent reaction of an organism to what is otherwise an innocuous protein. But it is our belief that once an organism becomes sensitized to an innocuous protein the response to this protein is remarkably similar to the results of an injection of crotalin. It is believed at present that the violent reaction of an organism to an innocuous protein is due to the accumulation in its cells and humors of a certain concentration of precipitin. It is, therefore, of interest to note that there is normally present in the blood of a dog a considerable quantity of precipitin for crotalin. This perhaps explains why crotalin is invariably poisonous, and proteins like egg albumin and horse serum require a preliminary period for sensitization.

If one arranges a series of Wassermann tubes containing 1 cc. of normal dog serum, and adds to each tube varying quantities of crotalin beginning with 0.01 mgm. and ending with 1 mgm., one obtains, on incubating these tubes for eighteen hours, a definite amount of typical precipitin in each tube. The control tubes are clear. The precipitin is manifested by mere turbidity in the first tube, and by flocculation in tubes containing 0.1 mgm. or more. When dogs are immunized to crotalin by repeated daily intravenous injections of a small dose, the precipitin titer of the blood rises. Far from using our data as evidence for the anaphylotoxin theory, our experiments can much more justly be urged in support of the Dale-Besredka precipitin theory of anaphylactic shock. Whenever a substance is introduced into the blood stream which, on access to the cells of the organism, evokes precipitin, the resultant upset causes the group of symptoms which we recognize as anaphylactic shock. If the precipitation is confined to the blood stream by the presence therein of an excess of precipitin, shock does not result. It follows as a corollary to this reasoning that the organism should invariably be protected from crotalin shock if the precipitin titer of the blood can be raised sufficiently.

Manwaring, Marino and Boone (1927) showed that, following a latent period of three-fourths of a minute to one minute, the injection of horse serum into a sensitized dog results in a fall in blood pressure and a rise in pressure within the bladder. They considered this rise of pressure as a characteristic response of the dog during anaphylactic shock. The injection of crotalin into a dog invariably has, as an accompaniment of the fall in blood pressure, a marked rise in intravesical pressure (fig. 4). The

mechanism of this increase in pressure is not clear. Dale and Laidlaw (1910 to 1911) showed that the depressor action of histamine was followed by a rise in pressure within the bladder. They stated that this rise was dependent on anemia of the sacral cord, and did not occur following its ablation. Whether this is the mechanism of the change of pressure in the bladder in anaphylactic shock and crotalin shock, we are not prepared to state.

One of the methods of determining the sensitivity of a human being to a foreign protein is to inoculate a small quantity of it intradermally. The individual is said to be sensitive when the [illegible] area shows reddening, whealing and surrounding arteriolar [illegible]. Following similar inoculation of histamine, as is well known, there [illegible] immediate reddening, whealing, and a reflex arteriolar flare. In view of the similarity of reactions common to anaphylactic and crotalin shock, we studied the effect of crotalin on intradermal inoculation into the skin of human subjects.

It was found that if a 1:500 solution of rattlesnake venom was similarly introduced into the skin there occurred the identical threefold reaction: reddening, whealing and a surrounding arteriolar flare. This result has been invariable. There has also been a tendency for the needle punctures to show as blood-stained points. However, it has been impossible to distinguish the results of inoculating 1:500 venom from what occurs following a similar inoculation of 1:10,000 histamine.

SUMMARY AND CONCLUSIONS

Anaphylactic shock and crotalin shock have points in common which are commented on in relation to two current theories of anaphylactic shock:

1. There is a sharp fall in blood pressure, accompanied by initial constriction of the splanchnic viscera, followed later by their marked engorgement. The blood often loses its coagulability.

2. The intradermal injection of crotalin is followed by a wheal surrounded by an arteriolar flare. The reaction is identical with that following a similar inoculation of histamine or of antigen into a sensitive patient. With inoculation of crotalin, there is rather constantly a tendency for the point of puncture to persist as a speck of blood.

3. The injection of crotalin into a pithed and artificially ventilated guinea pig, the movements of the lung of which are being registered on a smoked drum, is almost invariably followed by evidence of bronchiolar constriction, which is occlusive in about a third of the cases, so that the lungs are rigid in spite of considerable increase in the inflation pressure.

4. The excised uterus of the virgin guinea pig, perfused with Ringer-Locke's solution, shows maximal contraction when crotalin is added to the perfusing fluid.

5. When crotalin is incubated in small quantities with dog serum, typical

precipitation results. It is possible that this explains the anaphylactic-like effects of injecting crotalin intravenously. The blood of dogs immunized to crotalin by repeated injections consistently shows a higher precipitin titer. Possibly this explains the immunity, the precipitin in the blood fixing the venom before it gets to the cells of the body.

6. Following the administration of crotalin, there is a constant rise in pressure within the bladder of the dog.

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THE PHYSIOLOGIC ACTION OF RATTLESNAKE VENOM (CROTALIN)

VIII. A Comparison of the Physiologic Action of Crotalin and Histamine

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It is well known that extracts of various animal tissues on intravenous injection provoke a marked fall in blood pressure. The opinion has been current in recent years that the active ingredients are histamine and choline, in spite of the fact that such extracts, when injected into an etherized and atropinized rabbit, cause a fall in blood pressure. This should not be the case were histamine and choline the responsible factors. In support of the contention that histamine and choline are not the agents solely responsible for the depressor activity of tissue extracts, the work of Hanke and Koessler (1920) can be cited. They found that histamine-free peptone elicited a depressor response. The work of Abel and Kubota (1919) and of Best, Dale, Dudley and Thorpe (1926) has produced good evidence that enough histamine is present in tissue extracts to account for the major portion of their activity.

In this paper we are pointing out the histamine-like nature of an animal secretion which, although definitely not histamine, has many of the properties of this substance. A number of the reactions to crotalin and histamine are so strikingly similar that we are indicating the undesirability of relying solely on certain biologic reactions for detecting histamine. The increasing importance that is being assigned to histamine in various types of hypotension and in the reaction of the skin to various kinds of injury warrants full consideration of the facts.

The suggestion that the effects of crotalin are due to histamine is untenable. Prolonged extraction of the dried, alkalinized venom with absolute alcohol in the Soxhlet apparatus did not yield depressor substance.

The points of similarity between reactions to crotalin and histamine may be briefly stated as follows: 1, on intravenous injection into dogs and cats, each produces a marked fall in blood pressure which, when the dose is sufficient, may persist at shock level for an hour or more; 2, the plethysmographic picture accompanying the depressor activity of each

substance is almost identical; 3, the perfused uterus of the virgin guinea pig responds to each substance by maximal sustained contraction; 4, both substances when injected into guinea pigs produce bronchospasm; 5, both substances when needled into the skin of human beings in dilute solution produce reactions which are indistinguishable from one another; 6, each substance raises the blood pressure of a visceral organism, and 7, both substances cause a rise in intravesical pressure when injected into dogs (Dale and Laidlaw, 1910–1911).

The points of dissimilarity between the reactions of the two substances are: 1, the etherized rabbit invariably responds to crotalin by a fall in blood pressure; as is well known, histamine produces a rise in the blood pressure of such a preparation; 2, the post-mortem picture of animals killed by these substances is entirely different; 3, crotalin acts as an antigen; 4, histamine has no effect on the volume of erythrocytes of the dog, and 5, histamine does not impair the contractile power of skeletal or cardiac muscle (Dale and Laidlaw, 1910–1911).

In view of the fact that most of the outstanding reactions to histamine may be obtained with crotalin, we are suggesting the possibility that tissue extracts as well as other depressor substances in nature may depend, for their depressor activity, on a principle that is neither histamine nor choline. It is true that certain effects of tissue extracts can be attributed to the histamine which they contain. However, our observations on crotalin show that it is possible for an animal product that is free of histamine to manifest those reactions which are generally considered characteristic of histamine. It is usually admitted that a substance is histamine: 1, when it lowers blood pressure; 2, when it causes reddening, whealing and reflex hyperemia when needled into the skin, and 3, when it evokes contraction of the perfused uterus of the virgin guinea pig. Our observations have shown that these criteria are inadequate for the biologic identification of histamine.

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THE CONSTANT RATE OF ABSORPTION OF ETHYL IODIDE VAPOR AND ITS SIGNIFICANCE AS A BASIS FOR MEASURING THE CIRCULATION

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When a foreign gas or vapor is inhaled for 20 or 30 minutes, the rate of absorption generally decreases progressively as the blood and tissues of the body approach saturation. This is true of all those "non-reactive" gases which undergo no decomposition in the body and are eliminated unaltered and undiminished as soon as the air breathed no longer contains them. No method has as yet been devised, and perhaps none can be, to use a non-reactive gas for the estimation of the rate of the circulation of the blood, without requiring the active coöperation of the subject (Marshall and Grollman). Any method with such a gas is limited to mere laboratory use. It is necessarily vitiated by the brevity of the period (about 20 seconds) within which the absorption must be measured. It affords no steady state.[1] It does not take sufficient account of the saturation of the pulmonary tissues and the blood in the lungs, which according to Blumgart and Weiss is 21 per cent of the total blood in the body.

The method of Haldane, which makes use of the normal respiratory gases, has also the limitation of requiring the coöperation of a trained subject. Yet that method has the advantage over one with a non-reacting gas in that it measures a continuing and not merely a momentary and varying condition. Most of the recent studies along this line have used the device of the "virtual venous pressure of CO_2," introduced by Henderson and Prince. This device must be used very critically to give reliable results; and some of the discrepancies in the otherwise valuable work of those who now use it (Bock, Dill and their collaborators) are probably due to neglect of the precautions which Israëls and Lamb have recently pointed out. With care and on trained subjects this device affords the closest approximation to direct application of the Fick principle to man that has yet been devised. It has afforded much general information. Most important here, it gives values of essentially the same magnitude as those

[1] The expression "steady state" is here borrowed, but in a slightly altered sense, from Bock, Dill and Talbott.

by the ethyl iodide method. This fact strongly supports the general reliability of both of these widely dissimilar methods, for on animals measurements of the circulation by the Fick procedure and by the ethyl iodide method give concordant figures.

Ethyl iodide affords up to the present time the only vapor or gas with the characteristics essential to a method of measuring the circulation free from the limitations above metioned, and therefore universally applicable. It is the only method that has come into considerable clinical use, and that is today, after four years' experience, employed satisfactorily on patients in several clinics in various countries. In the considerable literature upon this method the fact has been established by critics, as well as supporters, that when a small, but constant amount of iodide vapor mixed with air is inhaled, the rate of absorption (the difference in the amount in inspired and expired air) from about the fifth, or at latest the tenth, to the twenty-fifth minute is constant, or decreases quite inconsiderably. Only one other factor is needed to determine the circulation, namely, the amount of ethyl iodide taken up by each liter of blood passing through the lungs. It is over the determination of this factor that objections have arisen.

Some of these objections can now be definitively eliminated, while one still requires to be met and so far as present data allow will be met in this paper. These objections have to do respectively with (1) the analysis of ethyl iodide by means of iodine pentoxide, (2) the automatic sampling of alveolar air, and (3) the coefficient, or effective coefficient, determining the passage of ethyl iodide from the alveolar air into the blood in the lungs.

Analysis by means of iodine pentoxide. The doubt which some physiologists have expressed, as to whether ethyl iodide vapor can be estimated by means of iodine pentoxide, appears to arise largely from the assumption on their part that the method was invented for this purpose by Henderson and Haggard. In fact, however, iodine pentoxide as a reagent for oxidizing and estimating organic gases, particularly carbon monoxide, has been long and widely used by chemists. It is generally used not in such a way as to yield the exact number of milligrams or fractions of a milligram of iodine that quantitative oxidation would indicate on paper. On the contrary chemists generally use it exactly as Henderson and Haggard, and as Mobitz and his co-workers have used it: namely, to give the relative amounts of the gas under analysis in a series of samples. For estimating the circulation this is all that is needed.

It is true that not all tubes of iodine pentoxide are reliable (Liljestrand and Zander). This is the case if in the filling of the tube the glass wool is touched by the fingers or organic material is otherwise introduced. Even a good pentoxide tube may vary unless conditioned from time to time by a period of overheating. But a tube of pentoxide is reliable for the purposes here involved, when it meets the following requirements: A small

amount of ethyl iodide is dissolved in water or saline. One cubic centimeter of this solution is drawn into a hypodermic syringe and injected into a wash bottle through which a stream of air passes. The stream of air volatilizes the ethyl iodide out of the solution, and is then drawn through the pentoxide tube and on through a solution of potassium iodide to collect the iodine liberated by the reaction between ethyl iodide and iodine pentoxide. Next an equal quantity of the solution is injected into the wash bottle slowly drop by drop, and its iodide is drawn through the pentoxide tube with the same velocity of air stream as before. Then half as much of the solution is employed. Then a quarter as much. If the pentoxide tube is reliable the amounts of iodine liberated in the four tests stand in the relation $1:1:\frac{1}{2}:\frac{1}{4}$. The quantities are measured in terms of the thiosulphate solution with which the liberated iodine is titrated. In our opinion the pentoxide method of analysis is not only simpler to apply, but also gives smaller errors than the silver nitrate method of Starr and Gamble, or that of Lehmann.

Automatic sampling of alveolar air. The method of automatic sampling of alveolar air employed with the ethyl iodide method consists in the withdrawal of the last portion or each of a succession of expirations. Its validity was questioned by one inexperienced writer with no experiments of his own. The reliability of the device, when properly controlled by dead space determinations, seems to be now quite fully established. Even such careful critics as Starr and Gamble find it accurate; and its increasingly wide use testifies that this is the general experience. The device has, of course, limitations, especially during the vigorous breathing induced by physical exercise, but these limitations apply almost equally to direct sampling of alveolar air by the original Haldane and Priestley method. The most important contribution recently to the subject of the determination of the alveolar air is that of Aitken and Clark-Kennedy. They have determined the rate of increase in the CO_2 content of the alveolar air during the respiratory cycle in muscular exercise. Their paper shows that even when the volume of breathing is 5 to 10 times the resting value, automatic sampling gives a CO_2 concentration which is higher than the average throughout the cycle by only something more than 10 per cent. It follows therefore that during quiet breathing no considerable error is involved in using as true average alveolar air the last portion of full normal expiration.

Calculation of dead space as check on accuracy of analyses. Particular attention should be called to the importance of using the calculation of the dead space of the respiratory tract for carbon dioxide and for ethyl iodide as a check upon the reliability of any set of observations. If a single measurement is wrong, or a leak has occurred, this check gives warning of error. Unless the dead spaces for the two gases come to the same value

within one or two per cent, the entire group of observations should be discarded. Several of the critics of the ethyl iodide method have failed to report the use of this check or the figures from which it might be calculated. There is, therefore, no way of estimating the reliability of their work.

Fate of ethyl iodide in the body. Starr and Gamble have carried out an extensive critical study of the ethyl iodide method. So far from rejecting it—as some, who must have read their papers rather carelessly, seem to think—their work distinctly supports the main features of the method. They state expressly that "ethyl iodide in conjunction with the Fick principle may be used for consistent and, we believe, reliable calculations of the cardiac output in man." They propose their technique merely as "an improved method for the estimation of blood flow in man by ethyl iodide," and describe their apparatus as modified from that of Henderson and Haggard. In these statements there is certainly no "rejection" or "refutation" of the ethyl iodide method, as some writers seem to wish to infer.

On certain points, however, in our opinion, Starr and Gamble reach erroneous conclusions. They estimate the rate of decomposition of ethyl iodide in the body at only 13 per cent per minute. On this ground they propose to add to the procedure of the method a "venous" determination by means of a rebreathed sample. This rate of decomposition seems to us, however, for reasons to be given below, very much less than the probable value. The addition of a rebreathed sample to the technique of the method adds somewhat to its complexity. It requires either a prolonged period of rebreathing or the coöperation of the subject. The addition of another factor renders the calculation of the circulation much less precise. On this account it has been avoided by Henderson and Haggard and by Mobitz and his collaborators. The determination of the gases in the venous blood by rebreathing is a matter for caution. It is not always reliable for CO_2, as Israëls and Lamb have recently shown. It is probably impossible for oxygen, for the venous tension is too far from the arterial to be reached in the time of rebreathing. It is difficult to make it precise for foreign gases, for at the beginning of the rebreathing the lungs are saturated with the gas.

The original formula of the ethyl iodide method for the calculation of the circulation was $\frac{(I-E)\times R}{A\times 2}=C$. The formula which Starr and Gamble propose, (omitting here some of their minor factors), is: $\frac{(I-E)\times R}{(A-r)\times K}=C$. In the first of these formulae the error of the denominator is proportional to A, the alveolar content of ethyl iodide. In the second it is proportional to the algebraic sum of A and r, r being the content of ethyl iodide in the rebreathed sample. The error of the method thus becomes (inversely)

proportional to the difference of two quantities each more or less inexact, instead of proportional merely to the more exact of the two quantities.

The question whether or not this additional factor is necessary turns upon whether or not it is better to estimate as a fixed factor, or to try to measure, the amount of ethyl iodide returning in the venous blood. Henderson and Haggard inferred from the rapidity with which the steady state is reached that the disappearance of ethyl iodide in the body is practically instantaneous and that no appreciable amount returns in the venous blood. It is evident now that the rate is not so rapid. But a rate of decomposition of 13 per cent per minute, as calculated by Starr and Gamble, is too low to account for the following facts, none of which appear to be doubted, and some of which are strongly supported by Starr and Gamble. These facts are: 1. Within 5 minutes, or at most 10 minutes, after the inhalation of ethyl iodide is begun the (approximately) steady state is reached. For 20 minutes thereafter the difference between the inspired and expired concentrations remains constant, or nearly so; as does the alveolar concentration also. 2. After the period of inhalation is ended the subject throws off through the lungs only about 4 per cent of the amount of ethyl iodide absorbed. 3. The remainder of the ethyl iodide is decomposed in the body, and excreted quantitatively through the urine during the next day or two as iodides combined with inorganic base.

It seems to us impossible to reconcile these facts with the view of Starr and Gamble that the rate of decomposition is only 13 per cent. If this were the true rate much longer periods, a half or three-quarters of an hour at least, would be required to reach the steady state. The amount exhaled after the inhalation is terminated would be a large percentage, instead of only a small percentage, of the amount absorbed. The amounts in the venous blood should be at least 200 per cent higher after an inhalation of 28 minutes than after only 3 minutes; instead of being, as Starr and Gamble report, an average of only 33.9 per cent higher with variations from 11.5 to 55 per cent for the subject S, and from 29.2 to 40 per cent for G. We are the more inclined to question the conclusion of Starr and Gamble on this point, as possibly based upon analytical errors, because calculation of the dead space for subject S in the experiments of March 15, in successive determinations, comes to such discrepant values as 0.217, 0.268, and 0.179.

In their experiments on dogs also the figures which Starr and Gamble report, based upon their method of analysis of the blood, work out to extremely improbable results. We calculate that they require the volume of the respiration to be 180 times greater than the circulation in one experiment and 11.5 times as large in another experiment. One of these figures is wholly impossible; the other is so unlikely as to suggest analytical errors as the probable explanation.

It is, however, an ungrateful task to calculate discrepancies in the data of a difficult investigation by able and conscientious investigators. We proceed, therefore, to report observations made by one of us (Mobitz) in collaboration with Lorenz, which indicate that the rate of disappearance of ethyl iodide is so rapid, once the steady state is attained, that only about 20 per cent of the amount taken up in the lungs returns in the venous blood in a form to oppose the taking up of an additional amount. This fact, in conjunction with the data reported by Starr and Gamble, seems to us to indicate that during the steady state a considerable amount of ethyl iodide is continually held in the blood, both arterial and venous, in some such combination as Henderson and Haggard have suggested. The following observations demonstrate that 80 per cent of the ethyl iodide absorbed from each breath disappears before the blood returns to the lungs.

The partial pressure of ethyl iodide in the venous blood returning to the lungs. In a glass syringe of 20 cubic centimeters capacity, 2 cubic centimeters of ethyl iodide, 12 cubic centimeters of physiological salt solution and 6 cubic centimeters of air are shaken vigorously for 2 or 3 minutes at room temperature. The air and the portion of ethyl iodide that has not gone into solution are then ejected from the syringe. The result is a salt solution which contains in each cubic centimeter, as analyses have proved, approximately the same amount of ethyl iodide as is absorbed in a minute per liter of respiratory volume by a man during an ordinary experiment. Lauter and Baumann have shown that the same magnitude of absorption per liter of respiratory volume applies to the dog and to man. A cannula of glass or nickel is introduced through the carotid artery into the aorta of a dog narcotized with morphine and urethane. The animal is also tracheotomized and the volume of breathing determined. For each liter of respiratory volume per minute, one cubic centimeter per minute of the solution of ethyl iodide is injected at a uniform rate into the aorta, and this injection is continued for 10 minutes. The animal is meanwhile inhaling air free from ethyl iodide, while its expired air contains an amount of ethyl iodide depending upon the concentration or rather tension (partial pressure) of the substance in the venous blood returning to the lungs. A dead space of one-third of the volume of the breath is assumed, and a circulation two-thirds of the respiratory volume, both of which are known to be approximately correct values.

The results of several experiments of this type are that the alveolar air under such experimental conditions contains about 20 per cent of the concentration of ethyl iodide that it does during an inhalation experiment. This fact demonstrates that only about 20 per cent of the ethyl iodide absorbed during an inhalation experiment returns to the lungs in readily diffusible form. The other 80 per cent disappears. Instead of a rate of decomposition of only 13 per cent per minute, as Starr and Gamble believe,

the rate is thus shown to be considerably more even in the fraction of a minute required for the blood to circulate once. The attainment of a steady state within 5 or 10 minutes, and its continuance for 20 minutes thereafter are in accord with a rapid, but not with a slow, rate of decomposition.

The volume of the circulation. Starr and Gamble, using rebreathed samples for estimation of the ethyl iodide content of the venous blood, calculate the circulation in man at values only about two-thirds as large as those obtained by most other recent investigators. Their values for the normal circulation would make the arterio-venous oxygen difference about 6 volumes per cent instead of about 4 volumes per cent, which is probably its correct value. The use of the value 2 for the coefficient of distribution in the original formula $\frac{(I - E) \times R}{A \times 2} = C$, results in figures for the circulation of a volume that would give an arterio-venous oxygen difference of about 4. For this reason we prefer, at least until further evidence is available, to retain the formula in its original form. But we recognize that in view of the experiments above reported the coefficient 2 is merely virtual and may be somewhat too low. It expresses, not the amount of ethyl iodide in the arterial blood, but the amount which during the steady state the blood takes up at each passage through the lungs, over and above the 20 per cent already in readily diffusible form and the larger amount held probably in some combination of a more slowly diffusible form.

Direct evidence on the amount of ethyl iodide that blood can take up during the time of a passage through the lungs is afforded by recent observations of Kaup and Grosse (1929). These investigators find, in accord with similar observations of Henderson and Haggard, that when blood (rendered non-coagulable with sodium fluoride) is equilibrated with air containing ethyl iodide for various lengths of time in vitro, it absorbs from all equilibrations up to about a minute an amount of ethyl iodide corresponding to a coefficient of distribution of the value 2. It is only after much longer equilibrations that larger amounts are found in the blood.

While the points which Starr and Gamble have presented certainly deserve careful consideration, the modification of the method which they propose has not yet been put to the test on patients. On the other hand many independent investigators in several different countries have found from extensive use that the ethyl iodide method, in its original form, gives on all types of persons results which agree well within themselves, and correspond closely with the relative vigor of healthy subjects and the clinical condition of patients. On this account it seems wisest to retain the simpler formula for purposes of calculation, at least until a better has been proved beyond question, and until additions to the technique have been fully tested on untrained persons both healthy and diseased.

CONCLUSIONS

Ethyl iodide is the only substance yet known which, when inhaled as gas or vapor, affords over a considerable time a nearly constant rate of absorption and a nearly constant concentration of convenient amount in the alveolar air. These two constancies afford peculiar advantages for measuring the flow of blood through the lungs. No method using a non-reactive gas can approach it in convenience and applicability alike to normal persons and to cardiac and other patients without requiring the coöperation of the subject.

The method of analysis by means of iodine pentoxide, when properly used, is reliable and accurate as well as simpler and more rapid than other methods. All methods of analysis for calculating the circulation should be controlled by estimations of the dead space.

Automatic sampling of alveolar air is now established as a reliable technique.

A rate of disappearance of ethyl iodide in the body of only 13 per cent per minute would not be compatible with the attainment of a steady state within so short a period as 5 or 10 minutes. Experiments here described show that the rate is actually about 80 per cent in a single round of the circulation.

The addition of a rebreathed sample complicates the technique of the method, particularly for clinical use, and increases the error of calculation. Although the coefficient used in the original formula for calculation of the circulation may be a little too low, it gives results which are of the correct magnitude, assuming that the relations are the same as they have been shown to be in the calf and the dog. This "virtual" or "effective" coefficient should be retained, particularly for comparative measurements of the circulation in healthy and diseased persons, until the validity of another value or a modified formula is proved.

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OBSERVATIONS ON THE SPINAL INTEGRATION OF STANDING

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For a period of years we have been able, in this laboratory, to maintain spinal dogs in excellent general health and, as is well known, this state is reflected in the marked reflex excitability of the spinal animal. One of our dogs, no. 10 (see appendix) was subjected to a complete dorsal spinal transection in April, 1925. Since that time the reflex activities of this dog have been under constant observation, and we became interested in the considerable ability developed by the isolated spinal centres of regulating the standing, stepping and running of the hind legs. When this dog is set free from its cage and allowed to wander about the room, it supports itself on its fore limbs and the shoulder muscles become very powerful as the result of such exercise. But, after a time, the hind legs which were at first dragged about by the dog showing phasic reflexes, began to support the hind quarters in a standing position for minutes at a time (fig. 1). Sometimes a swaying is the only feature which suggests the elementary nature of this posture. As soon as the animal moves off voluntarily, this posture is often upset, but frequently the hind legs step out, keeping no rhythm relative to the fore leg movements, but supporting the weight of the body in a staggering manner. Owing to faulty placement of the feet, slipping often occurs and the hind quarters will fall and be dragged along the floor. When the dog moves more rapidly, running and galloping of the hind legs have often been observed. This is a regular but awkward movement exerted with considerable power.

Such observations as these were somewhat surprising, since we had gathered from the literature that very little power of maintaining posture was to be expected from the segmental nerve centres. When we found that the standing posture was sometimes maintained for periods exceeding one hour, it appeared that this spinal component was more important than had been surmised, and an attempt was made to study the phenomenon. This paper forms an account of such observations.

GENERAL CARE OF SPINAL DOGS. Since the reflex excitability varies with the general health of spinal preparations, the maintenance of these animals

is important, and there are a few points in which others may benefit from our experience. Immediately following a complete spinal section, these dogs are, as is well known from Sherrington's (1906a) description, in a depressed condition due to spinal shock. During this period they are liable to pressure sores, local and general infective invasions, and cleanliness and careful nursing are necessary, if the animal is to survive and develop full reflex activity. As soon as possible, even before the dressings have been disturbed, change of position and exercise are the most helpful hygienic measures. We devised and improved a small wheeled cart into which the dog may be harnessed. The hind quarters are supported in the cart and kept from injury or dragging, while the dog exercises himself freely. In this way the appetite and general health of the animal improve early after operation, and a lot of the subsequent trouble from sores and infections is avoided.

Fig. 1. Photograph of spinal dog 10 standing.

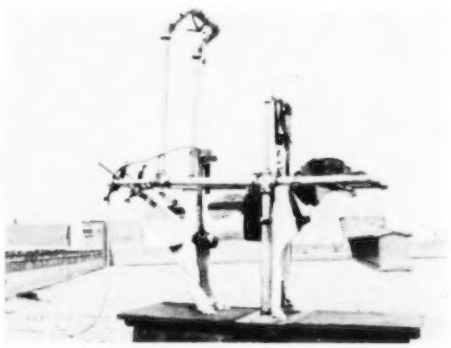

Fig. 2. Photograph of spinal dog 13 in frame (referred to in text).

THE EMERGENCE OF REFLEXES FROM SPINAL SHOCK. The appearance of the various reflexes out of the depression of spinal shock and their gradual improvement has been referred to by Sherrington (1906b). As will be seen from the appendix, in our dogs the knee jerk is the earliest reflex elicitable, the flexor and scratch reflexes later and still later the extensor thrust. Only after the lapse of some months were we able to observe any definite support of the hind quarters by the legs. On suspending the animal, the hind legs show the rhythmic stepping or mark time movements, but on placing the hind feet on the ground, the weight causes the legs to give way and the hind quarters sink. Later the placing of the feet upon a good surface leads to support, the mark-time continuing and finally after a few minutes the hind quarters sink. This standing improves greatly at a time 2 to 4 months after the transection, as will be seen.

METHODS OF RECORDING. In order to have the movements and standing of the hind quarters as free as possible from disturbance by voluntary movements of the head, neck and fore legs the dog was harnessed into a parallel bar frame (see fig. 2) lifted slightly so that the front paws are just above the level of the floor. The hind feet are then placed upon the platform supporting the frame which is covered with corrugated rubber matting so as to give a good foot hold. It is important to place the hind feet on the platform each time in the same position otherwise the angle at which the legs support the weight of the body varies. To insure this placement marks were made on the platform. The actively standing preparation when placed like this maintains the position with some swaying with the stepping movements. Too great a lateral movement of the rump leads to slipping of the feet, and in order to limit this lateral movement the semicircular guards seen in figure 2 were adjusted on either side of the rump. These do not hold the hindquarters in any way or prevent the stepping movements, but simply prevent too great a swaying. The step was in progress when the photograph (fig. 2) was taken, as can clearly be seen from the position of the right hind foot.

After many trials a comparatively simple arrangement was used to obtain graphic records of the standing and more especially of the sinking of the hind quarters. A rubber tube pneumograph (Harvard pattern) is placed around the animal's belly just in front of the thighs, forming a sling with a pad under the abdomen (refer to fig. 2). The chain attached to this pneumograph is hung from a spring balance which is fastened to a rigid cross bar extending over the frame. The height of the cross bar and the tension of the chain are so adjusted that when standing the dog receives little support from this sling. Attached to the tube pneumograph is a rubber tube which leads to a tambour recorder of large volume, writing on the blackened surface of a drum. The tube can be seen in figure 2, but neither tambour nor drum are shown. Figure 3 is a record of the spinal dog standing and stepping, short sections of tracing being taken at intervals of about 6 minutes. The upper trace is from the pneumograph sling which gives a record along the horizontal plane, with sharp wavelike rises and falls corresponding to the stepping movements. Time is recorded along the base line in 1 second intervals. Vertical lines on this tracing mark off periods of 20 seconds, and from this it can be seen that the stepping movements became slower in rhythm, at first 18 per minute and finally about 3 per minute. Finally the pauses or remissions between the steps become greater and the hind quarters eventually sink down. The tube pneumograph and spring balance are put under tension and extend, causing the recording stylus to fall toward the base line, as is shown in fig. 4. Readings of the spring balance give an estimate of the weight resting upon the sling. A calibration curve can easily be made by attaching a scale pan to

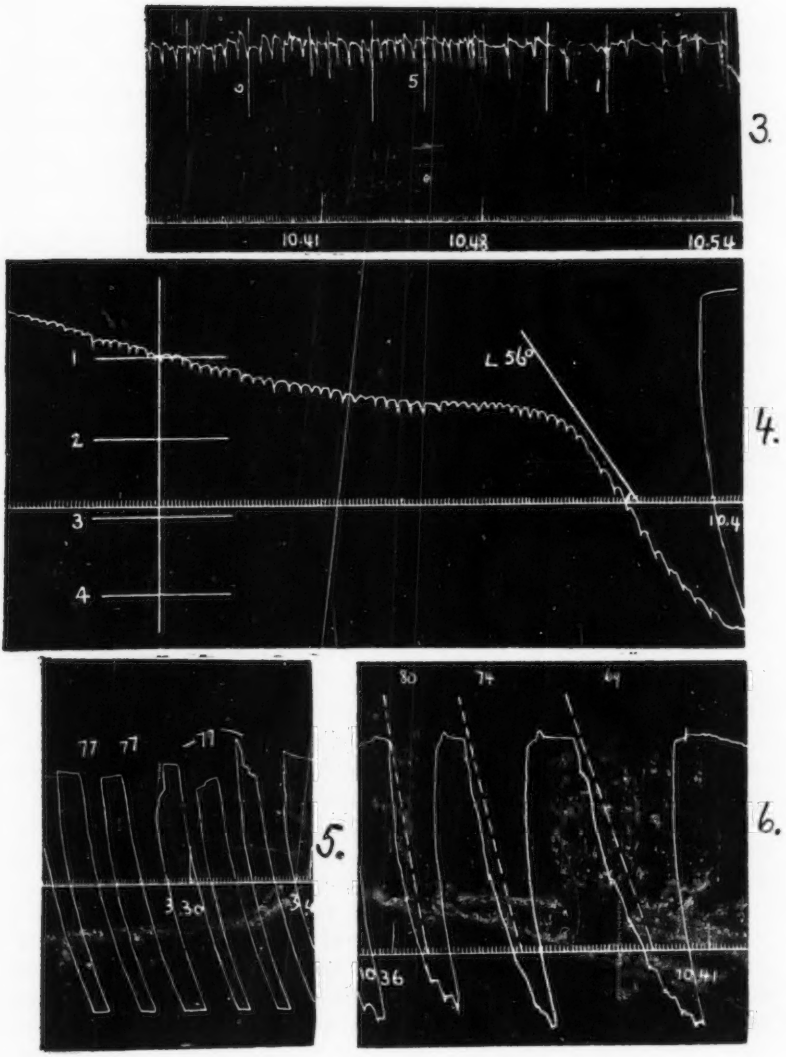

Fig. 3. Tracing recorded from standing spinal dog. Time in 1 second intervals. Jacquet chronograph.

Fig. 4. Record of sinking following a period of extension. Calibration curve on left. Angle of sinking indicated in degrees.

Fig. 5. Successive records following a period of flexion. Atonic type.

Fig. 6. Successive records following a period of flexion, showing tone recovery.

the tube pneumograph and adding weights. Such a curve has been drawn on figure 4 and shows the amount of extension for each pound. When the stepping movements cease the records show respiratory movements plainly, as can be seen in figure 4, and sometimes pulsations. If the animal were restless such records would be markedly irregular, since one cannot strap the anterior part of the body rigidly without causing discomfort. However, the suitable dogs soon learned to remain quiet in the frame and usually went to sleep soon after being placed in the harness.

Observations. At first all that was attempted was to find how long a time the spinal dog could stand under these conditions, and this time was found to vary considerably, as will be seen from the table of standing durations on different occasions (table 1). The shortest durations amount to a few minutes and the longest to well over an hour. The most frequent duration is from 20 to 35 minutes. Necessarily this time has been measured under conditions as uniform and quiet as possible.

When the graphic records of the sinking of the hind quarters were compared, it became apparent that another set of observations could be made, namely, on the rate at which the subsidence proceeded. After an animal has been standing and stepping for a period, it is noticed that the rhythm of the movement becomes slower, and remissions become noticeable (fig. 3). During these remissions the legs give way to the weight of the hind quarters which hang heavily on the sling, and another stepping movement brings about a recovery. Finally the stepping ceases and the curve inscribed falls slowly or rapidly down as more and more passive weight rests upon the sling and spring balance. If the angle of inclination of this curve with the base line is measured, a good idea of the rapidity of the collapse can be formed. Measurement of this angle forms a basis of comparison of curves taken under similar and different conditions as will be seen. Such angles are indicated on figures 4 and 6.

Results. *a. Duration of standing.* As has been mentioned above, this time varies between a few minutes and over an hour (table 1). The duration of standing is prolonged when the animal, on being set up, enters upon a brisk and vigorous series of steps, sometimes with other evidence of high tonus such as tremor. If the hind legs show an absence of the reflex stepping movements or tremor, the duration of standing is invariably short. The duration of standing therefore seems to depend upon the ease with which the phasic reflexes are elicited and therefore upon the general reflex tonus of the preparation. The tonic postural reflex is therefore sustained by activity of the phasic reflexes. The conditions to which the muscles have been subjected prior to observation are important and have been noted carefully. If the dog has been left in its cage before being placed on the frame, the hind legs have been allowed free stretching movements, and this usually results in prolonged standing. If the dog has been exercised

TABLE 1

The following measurements are given in the columns of table 1 from left to right: (1) date; (2) time of observation; (3) duration of standing in minutes; (4) the angle of inclination with the base line in degrees (as indicated in fig. 4) of the first collapse after standing. (5) angle 2. The angle in degrees of sinking after dog is put up in the manner described from a position of flexion (squatting posture). Successive angles are noted in the table so as to show whether or not tone recovery took place (cf figs. 5 and 6). (6) angle 3. The sinking angle in degrees after the dog has been supported with hind legs well extended for 5 minutes. In the earlier observations massage was used, as noted in the last column (cf. fig. 4).

DATE	TIME	DURATION	ANGLE 1 DEGREES	ANGLE 2 FLEXION	ANGLE 3 EXTENSION	
			Dog—Fox			
1927		*minutes*				
July 20	a.m.	30		60. 55. 60		
	p.m.	20		75. 84. 85		
July 22	a.m.	37	30	73. 71. 70	53	Massage
July 26	p.m.	18	55	60. 52. 50	49	Massage
				63. 65. 67		
July 27	p.m.	41	35	74. 71. 74		
July 29	a.m.	36	43	74. 78. 79	57	Massage
	p.m.	15	55	76. 75. 78	73	Massage
				81. 81. 82		
Sept. 27	a.m.	35	35	72. 69. 71	55	Massage
				70. 69. 64		
Oct. 7	a.m.	39	20	62. 62. 59	48	Massage
	p.m.	37	35	48. 49. 57	43	Massage
Oct. 22	a.m.	18	15	69. 70. 69	37	
Oct. 25	a.m.	60	48	73. 71. 70	60	Massage
	p.m.	16	63	78. 77. 76	63	
Oct. 29	a.m.	70	35	66. 63. 60	43	Standing
				68. 64		
Nov. 9	a.m.	32	35	58. 55. 53	48	
	p.m.	21	56	69. 63. 63	48	
Nov. 16	p.m.	11	33	68. 65. 63	38	
Nov. 23	p.m.	35	38	78. 70. 68	48	
Dec. 10	a.m.	30	47	79. 76. 72	42	Massage
1928						
Jan. 4	p.m.	2	35	82. 80. 80	27	
Jan. 10	a.m.	18	32	77. 66	29	
Jan. 17	a.m.	31	35	78. 74. 77	31	
Jan. 24	a.m.	2	32	80. 80. 82	28	
Jan. 28	a.m.	33	39	74. 39	20	
Feb. 4	a.m.	4	22	61. 55	23	
Feb. 25	a.m.	31	28	62. 58. 49	31	
Mar. 7	p.m.	30	22	51. 34	28	
Mar. 28	p.m.	6	30	73. 64. 52	31	
June 4	p.m.	22	24	73. 69. 73	28	

TABLE 1—*concluded*

DATE	TIME	DURATION	ANGLE 1 DEGREES	ANGLE 2 FLEXION	ANGLE 3 EXTENSION	
Dog—Fox						
1928		*minutes*				
July 11	p.m.	40	27	62	38	
Oct. 10	p.m.	64	20	63		Forcible flexion
Oct. 18	a.m.	8	50	74	67	
Nov. 7	a.m.	62	70	74. 78	70	
Dec. 28	a.m.	1	76	76. 74. 73	67	
1929						
Jan. 9	p.m.	25	52	74. 70. 64	55	
Jan. 16	p.m.	35	50	71. 54	52	
Jan. 22	a.m.	60	54	67		
	p.m.	16	61	76. 72. 73	49	
Feb. 27	p.m.	5	62	78. 75. 64	50	
Mar. 27	p.m.	3	65	77. 77. 77	54	
May 6	p.m.	25	62	80. 77. 75	64	
Dog—B & W						
1928						
June 5	a.m.	10	42	76. 69. 69	63	
June 8	p.m.	3		71. 81. 78	68	
June 15	a.m.	16	55	69. 65. 65	60	
June 25	a.m.	36	66	68. 70	60	
June 30	a.m.	5	75	75. 76. 78	53	
July 9	p.m.	17		73. 75	55	
July 20	p.m.	1	53	78. 74	69	
July 26	a.m.	20	68	78. 78. 79	45	
July 31	a.m.	15	55	76. 72. 67	51	
Oct. 4	a.m.	25	45	72. 75	65	

in the wheel cart for a length of time before observation, the standing time is shorter than average. The reason for this lies in the fact that the legs have to be tucked into the cart in the flexed position and this predisposes to a less active standing. Like all other reflexes this phenomenon showed a marked dependence on what had gone on before, and we have consequently been very careful in drawing conclusions as to tonus from the standing duration, unless the conditions are taken into account. The general state of the animal is indicated by the power of standing. When the dogs are upset and below par, the reflex excitability is lowered and the standing is poorly maintained. Visceral reflexes arising from feces, colicky pains, the state of "heat," et cetera, also cut short the time of maintenance, by inducing a squatting posture.

b. The rate of sinking as judged by the angle of curve with the base

line as indicated in figure 4 also varies over a wide range, and considerable attention was paid to the conditions responsible for this variation, since it is desired to use these curves as a basis for comparison of tonus. A steep curve approaching the vertical arc of the pointer (angle about 80°) is taken to indicate little or no tonus in the muscles opposing this sinking. A more gradual slope with an angle, sometimes as small as 20°, is interpreted as meaning considerable opposition to the fall, and therefore marked tonus. In the latter "tonic" type of curve the rate of fall is not by any means uniform. These curves (fig. 4) show first a very gradual slope, a definite shoulder, followed by a steeper fall which flattens out as the full weight hangs upon the sling. The spring balance shows the gradual slope to occur between 1 to 2 pounds; shoulder at 2 to 2.5 pounds; steep slope 2.5 to 4 pounds, tailing off at between 4 to 5 pounds. All grades of curve, between an "atonic" steep even line (fig. 5) and a "tonic" shouldered curve (fig. 4) have been obtained. It is possible to deduce from the shape of the curve and its steepest angle what conditions have preceded the record. If the animal's hind legs are passively or forcibly flexed and kept in that position for a few minutes, then set up in standing posture, the sinking will be rapid and an "atonic" type of curve will be obtained. The type of record indicating increased tonus is obtained under the following conditions:

1. The animal is placed in the standing position in the following manner. The hand is placed under the belly lifting up the hind quarters gently until the feet are just above the platform. Then the feet are quickly adjusted on the marks and the weight is allowed to rest upon them. If a succession of these placements is made and the records taken as in figure 6, often each succeeding record shows an improvement of tonus in its more gradual slope. This is shown clearly in figure 6, which is not by any means the most accentuated of its kind. We have called this "tone recovery." The degree of this recovery is an index of the tonic state of the preparation and for this reason successive figures for the angles are given in many cases in table 1. The improvement occurs up to a certain point and usually not beyond this so that successive curves reach a uniformity. Sometimes, when the excitability is high, the animal will, on being raised into position, stand and step for long periods of time before sinking down again. It is evidently the stretching of the legs and the contact of the foot pads with the platform which acts as a tonus intensifier in this case.

2. If the hind quarters are supported by the hand under the belly and the hind legs extended by the other hand, a series of steps will start, and the vigour of these steps will depend upon the excitability of the spinal neurones. If the hind legs be drawn out in extension until they are almost horizontally in line with the body, we have seen three different types of response. The commonest is the step movement. There is also frequently seen a spastic extension of both legs, similar to the stretch a normal dog will

indulge in on rising from sleep. The third response is a jumping or galloping movement in which both legs are vigorously pulled up and shot out together, often with sufficient force to escape the observer's hand. After such extension, if the dog's legs are set in position and the record started, it is found that standing is maintained for some time. Stepping often commences and the hind legs will support the body for as long as 30 minutes. When, however, the legs do give way and sinking occurs, the records show a gradual or "tonic" curve with a marked shoulder (fig. 4). Successive records will then become steeper until a uniformity is reached. This form of stimulus—extension—has been in our experience the most effective way of producing an increase in tonus, as shown by the time of standing, or by the gradual slope of the curves. It does not seem to make much difference whether the step or stretch or gallop is elicited. The duration of the extension is important, however, as it seems to be related to the effect produced. We have made use of a five minute period of extension, so as to make comparisons possible.

3. If the hind legs are massaged, rubbing especially the extensor thigh muscles, the curves indicate increase of tonus. This stimulus does not seem to be as effective as extension and it is difficult to find any quantitative relation between the massage and the effect produced. No record is reproduced, but the general contour of such curves following massage is similar to figure 4, usually with less marked tonic effect.

Conditions which lead to decrease of tonus, as indicated by rapid sinking of the hind quarters and steep curves: After the animal has been resting in the frame with the hind legs in the ordinary passive squatting position, "atonic" records are always obtained when the dog is placed in the standing posture. The legs give way almost immediately and an almost vertical record results (fig. 5). When the excitability and tonus of the preparation are high, it is sometimes difficult to get the hind limbs into a passively flexed position, because spontaneous recovery takes place, and the hind quarters jump up again into the standing position. When this occurs it may be possible to decrease the tonus by steady forcible flexion of the hind legs, applied for a few minutes after which the hind legs usually remain passively flexed. A type of "lengthening reaction" of the antigravity muscles has been produced. Records of the sinking taken after this flexed posture will be steep, even slopes, usually almost vertical. In succeeding records the degree of "tonic recovery" gives an index of the condition of the preparation, as mentioned in section (1).

DISCUSSION AND SUMMARY

A method of estimating the standing power of spinal dogs is described, in that the duration of the standing may be timed and a graphic record made of the speed of sinking.

These measurements have been made in the case of spinal dogs some months after complete spinal transection. The observations extend over a period of years.

The importance of the spinal centres in the integration of a posture is considerable in the light of these observations. It is evident that these centres are, in the intact animal, very dependent upon higher control, since it is only after the lapse of some months of isolation that segmental integration returns to any marked degree.

Judging from the conditions which obtain when standing is maintained for a long period, there is a very close relationship between phasic and tonic phenomena. When the phasic reflexes are active the standing posture is well maintained, and when feeble the tonus of the legs soon gives way under the weight of the body. The phasic reflexes seem to support and enhance the power of standing in the spinal animal, perhaps by leading to the accumulation of excitable substance in the appropriate arc neurones.

Judging from the measurement of the angles of subsidence, it is clear that the posture of flexion makes the curve of descent a steep one therefore reducing tonus. This has evidently an effect opposite in type to the myotatic response. In the flexed or squatting position the antigravity muscles are put under tension and under such conditions the myotatic response would be an increased tonus of the muscles. Possibly the myotatic response is earlier in action and fades away as the flexed posture is maintained. Some evidence for this is found in Adrian's (1928) observations that the impulses from a stretched muscle decline in frequency after a time during a prolonged stretch. If the effect of flexion as observed here is to produce a "lengthening reaction," then such a reaction leaves in its wake a depression of tonus perhaps due to inhibition. Fulton (1926) discusses fully the relation between the stretch phenomena and shortening and lengthening reactions.

Full extension of the legs or massage of the muscles is found to increase the tonus as indicated by a slower sinking. Extension would diminish the tension under which the antigravity muscles operate and might therefore be expected to diminish the tonus. But extension and massage favour the development of the phasic responses, such as the stepping, jumping and stretch reflexes. It seems clear from these observations that this results in an enhancement of the standing tonus of the preparation. If it is to be considered as a species of "shortening reaction," such reaction results in accumulation of excitable material in the arcs, so that tonus is increased for a period of time.

It will be apparent from results such as are contained in table 1, that only a few observations or records taken in this manner might well be misleading as an estimation of muscular tonus. Conditions which lead to enhancement or depression of the tonus must be carefully taken into account.

When this is done the observations become of value as a basis for the comparison of standing power under different circumstances. Work has been in progress for some time on the influence of the sympathetic innervation and on the effect of drugs.

I wish to acknowledge the great assistance I have received from my technician, Mr. William Lawson, during this research. My thanks are due to Dr. M. J. Ormerod for help with many of the operations and experiments, and to Prof. V. E. Henderson for suggestions and criticism during the writing of this paper.

APPENDIX. *Description of spinal dogs.* The spinal dogs which have been used chiefly for these observations are three in number and an outline of their history follows:

No. 10. Salome. Airedale hybrid, about 1 year. Weight, 13 kilo. April 1, 1925. Complete spinal transection under ether. Level of thoracic IV.

Reflexes. Knee jerk—appeared on emerging from anesthesia. Flexor—Faint on April 2. Tail retraction. Crossed extension—April 5, first plainly visible. Scratch—April 5, faint response. Extensor thrust—inconstant about April 20. Reflex standing—some tonus on standing hind legs, about end of April—collapse very soon.

Muscular atrophy. At maximum about 2 months after operation. About 4 months steadily improved and in September, 1925, atrophy had practically disappeared and no sign of any lesion apparent externally.

General. During summer of 1925 gave birth to 8 pups, five of which were successfully raised by mother. Visceral reflexes with associated movements of tail, squatting of hind quarters noted on April 7, 1925. These became quite regular and we have never had trouble with retention of urine or feces.

Standing observations. After September, 1925 (six months after operation) standing on hind legs steadily improved and has become a surprising feature of this dog's behaviour. When put out on laboratory floor, the hind quarters will be supported for minutes at a time, if the dog can be persuaded to stay quiet (fig. 1). When the dog moves off, the hind legs will frequently step out, the feet being placed fairly well until some slip or awkward step results in swaying over of the hind quarters. These may right their position again and step along, the rhythm of the step having no relation to the front foot steps, excepting by coincidence. When the dog travels more rapidly the hind legs enter into awkward galloping movements quite powerful but showing frequent side slips on a smooth floor. Sometimes the galloping is carried out with tail lifting and wagging, and when the movement agrees in direction and rhythm with that of the fore part, surprising progress is made.

Graphic records of this dog's standing have been made, but she was soon found not so suitable for the frame as the smaller fox terriers, because she is restless, and struggles in the harness. The considerable weight of the hind quarters was also too great for the sling. For these reasons most of the records have been obtained from the other spinal dogs.

No. 13 Fox. Fox terrier, ♀. Chestnut and white; 8.3 kilo. February 24, 1927, complete spinal transection under ether. Thoracic IV.

Reflexes. Knee jerk appeared on emerging from anesthesia. Flexor reflex—faint four hours after operation. Tail response. Crossed extension—faint February 25th. Scratch—faint toe movement February 25th. Extensor thrust—faint but definite, March 10th.

General. Initial weight—8.3 kilo. November 1927—7400. January 1928—6700. February 1928—6600. June 1928—8000. October 1928—8500. January 1929—9400. June 1929—8800. Atrophy of muscles noticed soon after spinal section and weight went down until February 1928. Many thread worms were found and vermifuge administered. In April 1928 muscle atrophy began to lessen, and in June 1928 the muscles had filled out until weight was back to initial figure. No sign of atrophy at present. Dog was in excellent health in spite of loss of weight. Visceral reflexes noted in March, 1927, and have been regular since. No trouble from retention of urine or feces. The only gastro-intestinal disturbance caused by worms noted above.

Standing observations. In June 1927, four months after the spinal transection, the dog was noticed to enter into stepping movements and support the weight of hind quarters when placed on floor and kept quiet. The frame and harness were made and after sundry improvements, regular observations and records were made with this dog from July, 1927 until the time of writing. This dog soon became accustomed to being harnessed in the frame, and on being set up soon becomes quite quiet, often going to sleep during the observations. The records taken are for the most part smooth and regular, broken only by quiet respiratory movements. Most of the graphic records published are from this dog. Figures 3 to 6.

No. 14. B. and W. Fox terrier, ♀. Black and white. Initial weight, 8 kilos. March 1, 1928. Complete spinal transection under morphine and ether. Thoracic III.

Reflexes. Knee jerk appeared on emergence from anesthesia. Flexor R.—March 2, 1928. Feeble leg flexion and tail response. Crossed extension—March 5, 1928, plainly visible. Scratch—March 2, 1928, faint response. Extensor thrust—March 28, 1928, inconstant on left side, absent on right.

General. Atrophy of muscles apparent during first month after operation, but after this muscles rapidly filled out. In June 1928 weight was 8500 and atrophy scarcely noticeable. In October 1928 dog is in poor condition. Has torn operation wound in groin and large septic area is present. Dog killed. Post-mortem verification of site of transection of spinal cord. Parts of sartorius and gastroc. muscles sectioned and stained.

Standing observations. Some support of hind quarters with stepping of limbs on being placed in position, was noted toward the end of April 1928, about 2 months after operation. This improved steadily and the dog was used on the frame for recording in June 1928 and regularly until October 1928, when dog was destroyed. This dog similar in type of dog 13, was very suitable for the frame, remaining quiet in the harness during the observations.

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